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"IMMUNOGENICITY OF BIOLOGICAL DRUGS IN INFLAMMATORY RHEUMATIC DISEASES: CLINICAL RELEVANCE AND THERAPEUTIC DRUG MONITORING"

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To Germán and Sara

Acknowledgements

First, I wanted to dedicate this thesis to the patients because they are the main motivation of this work with the intention to improve the management of their disease and their quality of life.

I wanted to thank my family because they have always been at my side supporting my decisions. Firstly, some words for my mother and my sister who have given me an unconditional support to follow my life-path. To Gracibel and Paco, thanks to them this thesis has been able to be written. Finally, to Germán and Sara, two gifts of life.

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ABSTRACT

In the present thesis work, we show the data from a research line about the immunogenicity of biologics and its clinical relevance in rheumatic patients under biological therapy. This research was motivated due to the clinical observation that a significant percentage of patients lost treatment efficacy to treatment with biological drugs, despite an initial good response and that they continued with the same treatment regimen. Then, our purpose was to investigate which factors influenced on this treatment failure.

Our first studies were carried out in rheumatoid arthritis (RA) and Spondyloarthritis (SpA) patients under infliximab (Ifx) therapy. In these works, we demonstrated that lower Ifx levels and/or detectable ATI were associated with a poor clinical response. In addition, it was seen that patients receiving methotrexate (MTX) co-therapy less frequently developed antibodies to Ifx (ATI) or showed lower ATI levels than patients under monotherapy. Another important aspect was that ATI development was associated with treatment survival and with the incidence of adverse events such as infusion related reactions. The association between drug levels and clinical outcomes was also evaluated with other biological drugs such as golimumab and tocilizumab.

Later, we noticed that serum trough drug and anti-drug antibodies (ADA) monitoring to the first TNFi could have an influence on future therapeutic decisions. On the other hand, we saw that Ifx intensification strategies have a limited effect and are not associated with a significant clinical improvement.

We made a review on tapering or discontinuation strategies in SpA patients, concluding that tapering strategy is successful in SpA with low disease activity but discontinuation strategies are not recommended because they lead to flare in most cases. Two observational studies comparing the tapering strategy with the standard therapy regimen in RA and SpA patients were performed. Both show that tapering is feasible in clinical practice with similar clinical evolution than patients under a standard therapy regimen.

Finally, we carried out two studies demonstrating that adequate clinical monitoring together with tools such as drug monitoring support the treatment optimization, in order to treat patients more safely and with a significant reduction of costs.

RESUMEN

Esta tesis doctoral trata sobre la inmunogenicidad de las terapias biológicas y su relevancia clínica en pacientes con enfermedades reumáticas. Esta investigación fue motivada por la observación clínica de que un porcentaje significativo de pacientes perdía eficacia del tratamiento a pesar de una buena respuesta inicial y de continuar con el mismo régimen terapéutico. Entonces, nos propusimos analizar qué factores podrían influir en el aclaramiento del fármaco.

Nuestros primeros estudios se realizaron en pacientes con artritis reumatoide (AR) y espondiloartritis (SpA) tratados con infliximab (Ifx). Se demostró que los pacientes con niveles séricos más bajos de Ifx y/o presencia de anticuerpos anti-infliximab (ATI) se tenían una peor respuesta clínica. También se observó que los pacientes que recibían simultáneamente metotrexato (MTX) presentaban con menor frecuencia ATI en el suero o se detectaban niveles más bajos de ATI que en los pacientes en monoterapia. El desarrollo de ATI se asoció con una peor supervivencia de Ifx y con mayor incidencia de eventos adversos como reacciones relacionadas con la infusión. La asociación entre los niveles de fármaco y los resultados clínicos también se evaluó con otros biológicos como golimumab y tocilizumab

Observamos que la monitorización de niveles séricos de fármaco y/o anticuerpos frente al fármaco contra el primer TNFi podría influir en futuras decisiones terapéuticas. Por otra parte, vimos que las intensificaciones de tratamiento con Ifx tienen un efecto limitado y no está asociada con una mejora clínica significativa.

En una revisión sobre las estrategias de optimización o discontinuación en los pacientes de SpA objetivamos que la estrategia de optimización de dosis es factible en SpA con baja actividad, pero la estrategia de suspensión no se recomienda porque conduce al desarrollo de brotes en la mayoría de los casos. Se realizaron dos estudios observacionales que compararon la estrategia de optimización frente al régimen de terapia estándar en pacientes con AR y SpA. Ambos muestran que la estrategia de disminución de dosis es factible en la práctica clínica con una evolución clínica similar a los pacientes bajo un régimen de terapia estándar.

Por último, se realizaron dos estudios que demuestran que un seguimiento clínico adecuado junto con herramientas como el control de los niveles séricos de fármacos pueden

ayudar a que las estrategias de optimización se lleven a cabo de manera más segura con una reducción significativa de costes.

INDEX

ABBREVIATIONS

Aba: Abatacept

ACPA: Anti-citrullinated peptide antibodies

Ada: Adalimumab

ADA: Anti-drug antibodies

ADA +: Anti-drug antibodies positive

ADA-: Anti-drug antibodies negative

ANA: antinuclear antibodies

Anti-TNF: Anti tumor necrosis factor

AS: Ankilosing spondylitis

ASAS-20: Assessment in Ankylosing Spondylitis response criteria

ASDAS: Ankylosing spondylitis disease activity score

AU: arbitrary units

AUC: area under the curve

AxSpA: axial SpA

BASDAI: Bath ankylosing spondylitis disease activity index

BMI: body mass index

Certo: Certolizumab

CD: Crohn disease

DAS28: Disease activity score 28

DMARDs: Disease modifying anti-rheumatic drugs

ELISA: Enzyme-linked immunosorbent assay

Etn: Etanercept

ESR: erythrocyte sedimentation rate

EULAR: European league against rheumatism

Goli: Golimumab

HAMA: human antimouse antibodies

HACA: human antichimeric antibodies

HDQ: hydroxycloroquine

HAHA: human antihuman antibodies

HMSA: homogeneus mobility shift assay

HPE: high performance ELISA

iv: intravenously

LOCF: Last observation carried forward

NSAIDs: non steroidal anti-inflammatory drugs

nraxSpA: non radiographic axSpA

PsA: Psoriatic Arthritis

PIA: pH-shift-anti-Idiotype Antigen binding test

PICOS: Population, Intervention, Comparison, Outcome and Study design

PK: pharmacokinectic

polyHRP: streptavidin-polyperoxidase

RA: Rheumatoid arthritis

RIA: Radioimmunoassay

RF: Rheumatoid factor

RGA: reporter gene assay

ROC: receiver operating characteristic

Rtx: Rituximab

sc: subcutaneously

SpA: Spondyloarthritis

SpA associated to IBD: SpA associated to inflammatory bowel diseases

SSZ: sulphasalazine

TMB: tetramethylbenzidine

TNF: tumor necrosis factor

TNFα: tumor necrosis factor alpha

TNFi: tumor necrosis factor inhibitors

Tcz: Tocilizumab

vs: versus

INTRODUCTION

Efficacy of the biological inhibitors of TNF in rheumatic diseases

The rheumatic diseases such as rheumatoid arthritis (RA), seronegative spondyloarthropathies (SpA) and psoriatic arthritis (PsA) are chronic inflammatory disorders that lead to an important functional disability(1–3). The prognosis of these inflammatory disorders has changed considerably along with the introduction of biological drugs(4). Biological drugs are protein macromolecules whose active principle is produced or derived from a living organism and are made by means of recombinant DNA and / or controlled gene expression methods(4). The first biological drugs to be developed were directed to neutralize the tumor necrosis factor alpha (TNFα)(4,5) (*Figure 1*).

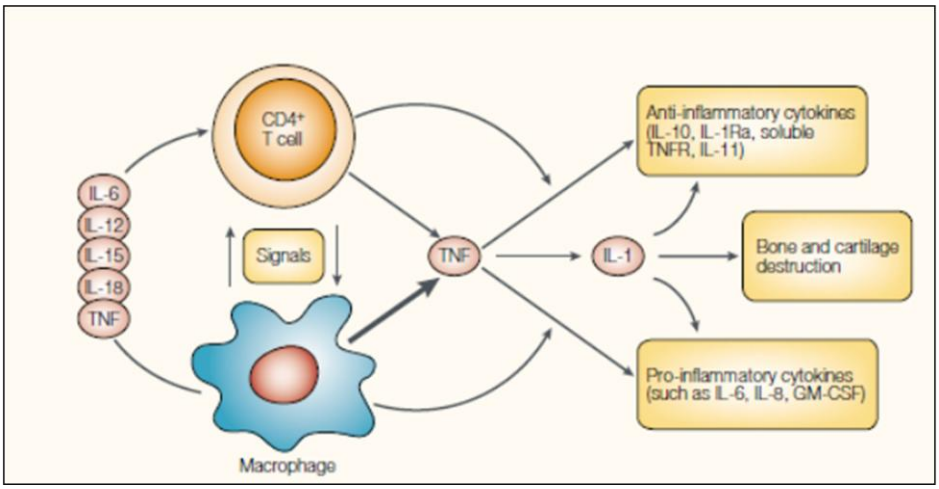

Figure 1. Scheme indicating that TNF is at the top of pro-inflammatory "cascade". Adapted from Feldman et al. *"Development of anti-TNF therapy for rheumatoid arthritis"*. Nature Reviews Immunology. 2002;2:365

Nowadays, there are five tumor necrosis factor inhibitors (TNFi) available, of whom three are monoclonal antibodies [Infliximab (Ifx), Adalimumab (Ada) and Golimumab (Goli)]. One is a pegilated $F(ab)_2$ fragment of a humanized monoclonal antibody Certolizumab (Ctz) and the other is a fusion protein between 2 molecules of the TNF receptor extracellular domain (p75) and the constant portion of a IgG1 immunoglobulin, known as Etanercept (Etn)(5–10).

Moreover, more biological drugs with another mechanism of action have emerged in the last years, such as Rituximab (Rtx), an anti-CD20 chimeric antibody(11); Abatacept (Aba), an anti-CD80/anti-CD86 fusion protein(12); Tocilizumab (Tcz), a humanized anti-interleukin-6 receptor antibody(13); Secukinumab (Secu), a monoclonal anti-IL17 antibody and Ustekinumab (Uste), a monoclonal anti-IL12/23 antibody(14–17) (*Figure 2*).

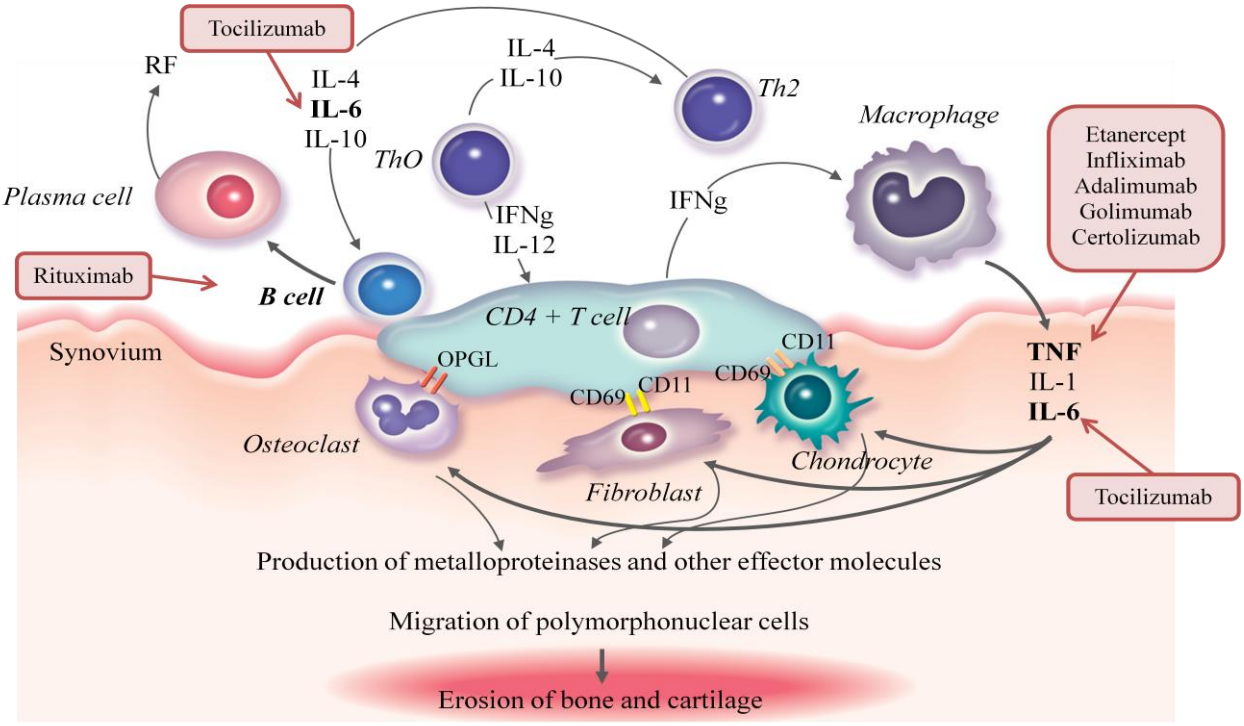

Figure 2. Scheme showing the major cytokines involved in the pathogenesis of rheumatoid arthritis. Adapted from Choy et al. *"Cytokine pathways and joint inflammation in rheumatoid arthritis"*. N Engl J Med 2001 Mar 22;344 (12):907-16

The efficacy of the TNFi biopharmaceuticals in the treatment of inflammatory diseases have been demonstrated in many studies, showing that a considerable proportion of patients under treatment with these drugs reach a lower disease activity or remission(5,6,8–10). However, some patients do not improve during induction therapy phase (primary response failure), while others present an initial good clinical response that is lost later on during treatment (secondary response failure). In rheumatic diseases such as RA under TNFi, it has been described that approximately one third of patients suffer a primary response failure(18). This situation normally

leads to intensifying therapy strategies with the consequent increasing therapy costs and risks of adverse events(18).

Up to 50% of patients with chronic inflammatory disease, such as RA or Crohn disease treated with TNFi eventually lose response to the biological drugs(19,20). The underlying mechanisms involved in the TNFi failure are not completely understood. Several factors can relate to this behaviour, like differences in the drug bioavailability and drug PK, as well as pharmacodynamic issues involving the basic mechanisms that drive inflammation in the affected tissues(18).

These findings have motivated a great scientific interest in the last years that attempt to define factors that can affect the bioavailability and pharmacokinetic (PK) of the biological therapies in order to use the most effective therapeutic strategies in patients with chronic inflammatory diseases(21–27).

Influencing factors on the efficacy of the biopharmaceuticals

As a result of research in the recent years, some factors are known to influence the biological drug efficacy. Among the main factors are the body mass index (BMI), smoking habits, serotype (in RA patients), serum trough drug levels and the concomitant therapy with disease modifying anti-rheumatic drugs (DMARDs)(28–32) (*Figure 3*).

RA patients with high BMI seem to have a poorer clinical response. Adipose tissue itself has pro-inflammatory characteristics, which in addition to the disease activity negatively influence the therapy outcome in RA patients(33). In the METEOR cohort(28), obese RA patients were significantly less likely to be in low disease activity. Similar findings were observed in an early RA cohort (CATCH study), where an inverse relation between increased BMI, from overweight to obesity, and the likelihood of achieving remission was seen. In the GISEA registry, obese RA patients under TNFi were significantly more likely to fail reaching remission at one year (28).

Smoking habit has demonstrated to be associated to extra-articular manifestations, radiographic progression and clinical response to DMARDs in RA patients. Similar correlation has been shown between smoker RA patients and TNFi treatment response(34,35). In a Swedish

early RA cohort, it was proven that current smokers had less chance to achieve a good European league against rheumatism (EULAR) response in comparison with never smokers [independent of Anti-citrullinated peptide antibodies (ACPA)status](36). The reasons to encourage rheumatic patients to drop out smoking are numerous, including to have less adverse effects along the treatment, less cardiovascular co-morbidities and the likelihood of a good response to DMARDs and TNFi(36).

In the British registry, it has been reported that seropositive [positive rheumatoid factor (RF) or ACPA] RA patients present a lower clinical improvement (measured by delta-DAS28) than seronegative RA patients(37). Nevertheless, contradicting studies later emerged regarding serotype and clinical response(38). In a recent meta-analysis including more than 5000 RA patients, an overall lack of an interaction between serotype and outcome with TNFi is shown. On the other hand, a higher likelihood of achieving EULAR response in seropositive patients has been described with another biopharmaceutical, such as Rtx(38). In the ACTION trial, non-responding RA patients under TNFi who started Aba treatment showed that ACPA positive RA patients have a higher likelihood to reach the good EULAR response and a better retention on the therapy(39).

In several clinical trials, TNFi have demonstrated an enhanced effect when given in combination with immunosuppressive drugs like methotrexate (MTX)(40–43). The CONCERTO study regarding early DMARD-naïve RA patients proposes that a minimum MTX dose of 10 mg weekly should be co-prescribed with Ada to achieve a good clinical response(40). In contrast, no enhanced effect on efficacy was found in PsA when Etn in combination with DMARDs was compared with Etn under monotherapy(28). For other biologics such as Rtx or Aba in RA patients, the superiority of the combinations with MTX over monotherapy to reach a better clinical evolution has been proven(28). However, the concomitant therapy with MTX versus monotherapy does not seem to be more effective in RA patients treated with Toci(28).

The association between TNFi serum trough drug levels and clinical outcomes will be discussed later with more detail. Nevertheless, it is important to highlight that many factors can influence trough levels, including adherence to treatment, BMI, co-prescription of DMARDs and the formation of anti-drug antibodies(28). A high BMI was the strongest predictor of low drug levels in a prospective RA cohort treated with Ada or Etn(44). The effect of the co-therapy with MTX on drug levels and anti-drug antibodies (ADA) detection has been described in several publications in rheumatic patients(25,45–50). In addition, the dose-dependent effect of MTX in

preventing the ADA appearance in RA and PsA patients treated with Ada has been described(46,50).

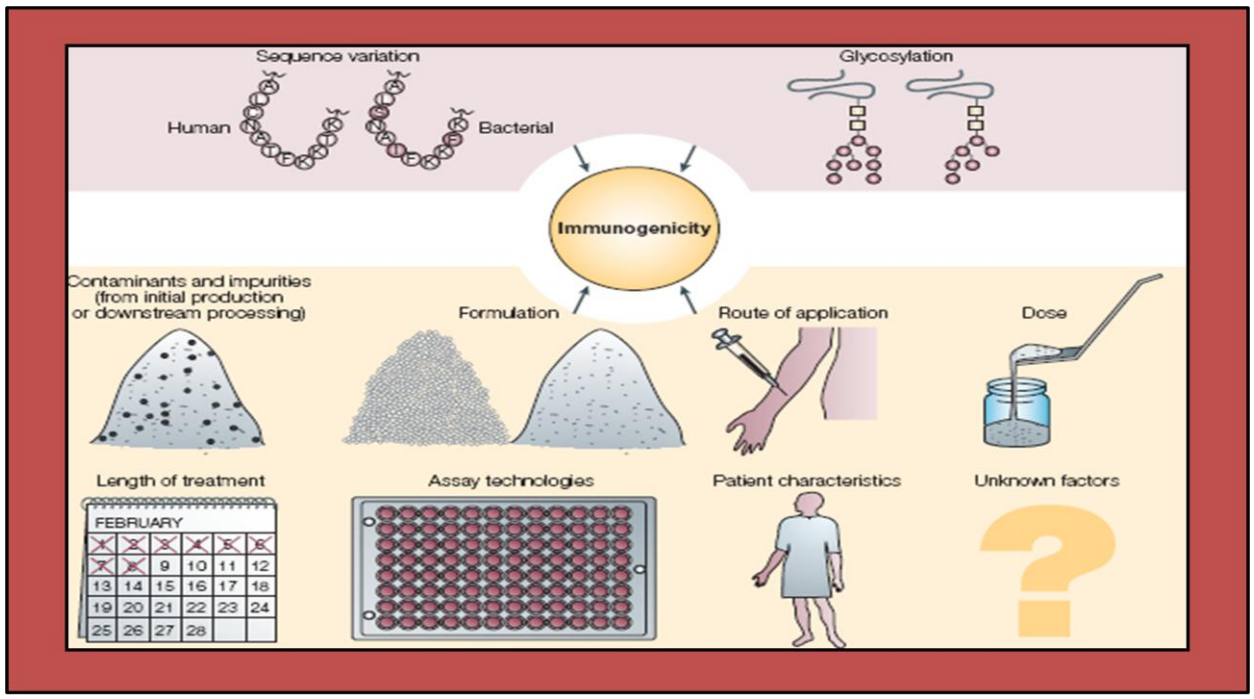

Figure 3. Scheme indicating that TNF is at the top of pro-inflammatory "cascade". Adapted from Schellekens H."Bioequivalence and the immunogenicity of biopharmaceuticals. Nat Rev Drug Discov 2002:457-62.

Immunogenicity of tumor necrosis factor inhibitors

Immunogenicity refers to antibody formation against a certain drug and all biological drugs can induce an unwanted immune response, mainly depending on its constitution(49,51–58). The immune response against native biologics like human hormones, growth factors and cytokines occurs only when the natural tolerance is broken and differs from the immune response against designed biologics containing new foreign epitopes(54,56,58). Chimerical drugs have a higher capacity of inducing immunogenicity as compared to humanized and to completely human drugs(53,54,56). Immunogenicity of fusion proteins depends on their similarity to native proteins and Etn seems to have a less immunogenic structure compared with the other TNFi agents(54,56)

The first antibodies used in the treatment of human disease were of mouse origin, but most patients produced human antimouse antibodies (HAMA)(56). Immunogenicity of these antibodies was subsequently reduced by replacing murine constant regions with human ones, resulting in chimeric antibodies such as Ifx and Rtx, but they also show to induce the formation of human antichimeric antibodies (HACAs)(56,57). Humanization of the variable regions, like in Certo or Tcz, and the latter introduction of totally human antibodies further reduced immunogenicity, however, even fully human antibodies, like Ada and Goli, may induce the production of human antihuman antibodies (HAHA) which resembles the formation of anti-idiotypic antibodies to unique determinants in endogenous antibodies which is characteristic of the natural immune network(56). All of these antibodies are currently denominated anti-drug antibodies (ADA). The fusion part of some biopharmaceuticals may contain new epitopes that can be recognized as foreign by the immune system. Etn is a dimeric fusion protein consisting of two molecules of the TNF receptor 2, linked to the Fc portion of an IgG1 and has a less immunogenic structure compared with the other TNF-blocking agents(56). Only the fusion part of the molecule can contain immunogenic epitopes and the active portion of the protein, as part of a natural biological receptor, is seldom the origin of an immune response(56).

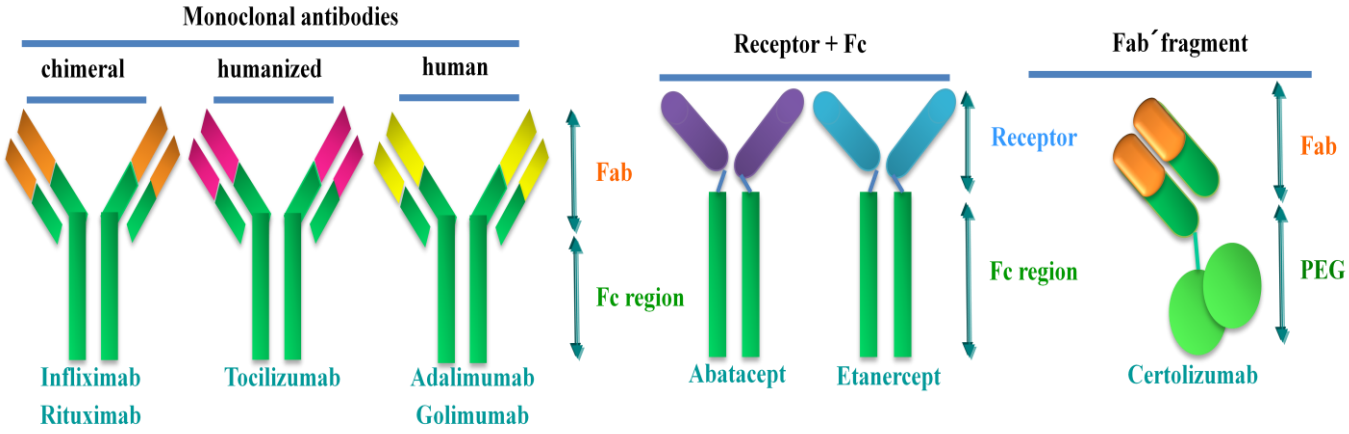

Figure 4. Molecular structural differences among the available TNFi drugs for rheumatic diseases

It has been published that the consequence of the ADA development is the alteration in pharmacokinetics(18,21–23,25,27,49,54,59). The ordinary half-life of IgG1 is around 3 weeks. Immune complexes clear faster from circulation(49,56,60). Over the last years, several reports

have shown that in the presence of ADA, biological drug serum levels are importantly reduced, suggesting the neutralization of the drug resulting from the immune-complexes formation(49,54,58,61–63) (*Figure 5*). ADA can form multivalent immune complexes with the target drug, leading to rapid clearance and/or inactivation of the drug, thus requiring dosage escalation or concomitant therapy with another agent(54,62,64,65). Rapid clearance of immune complexes may occur regardless of whether the antidrug antibodies neutralize the TNF-binding activity of the drug or not(54,62,64).

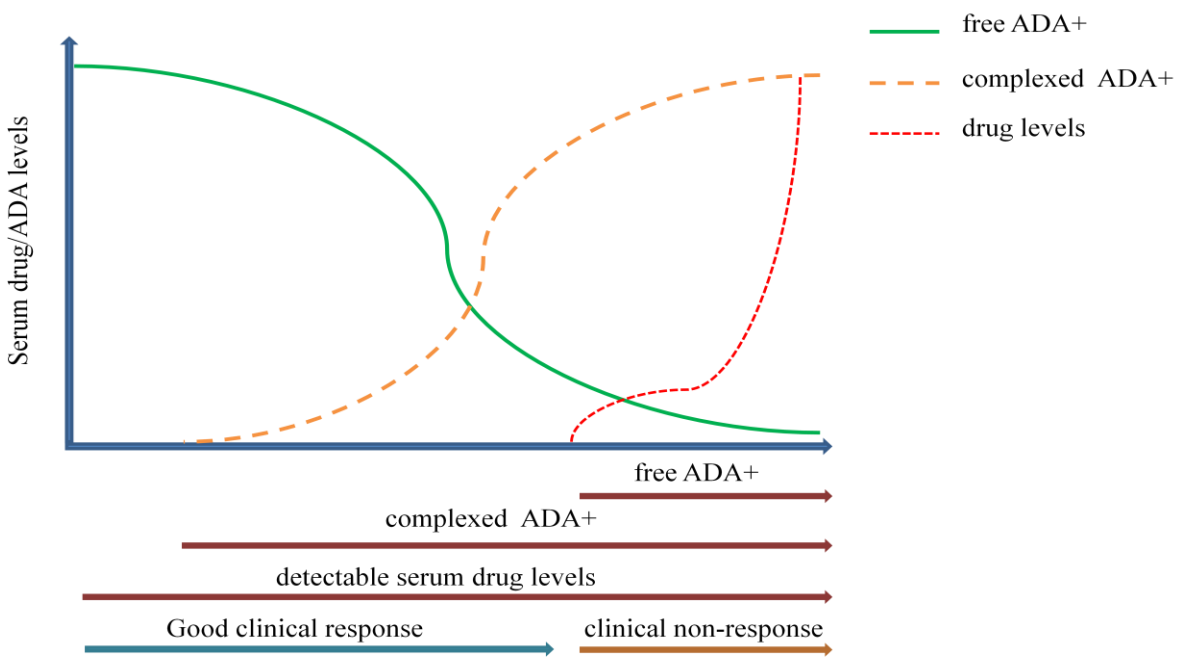

Figure 5. Serum concentrations of drug, free ADA and complexed ADA. Adapted from Schaeverbeke et al. "*Immunogenicity of biologic agents in rheumatoid arthritis patients: lessons for clinical practice*". Rheumatology August 12, 2015;53:299-309

Two different types of ADA have been described. Some studies show that at least some infliximab-specific ADA have neutralizing capacity(66). The dutch group has shown that at least 98% of adalimumab-specific ADA are capable of neutralizing this agent, however, binding of both neutralizing and non-neutralizing ADA to the therapeutic agent can result in the formation of immune complexes, which are then cleared from the circulation, reducing the drug's half-life(62). The effect of neutralizing or binding ADA on the clinical response to anti-TNF agents is dependent on the type of therapeutic agent(62,67).

Nevertheless, the prevalence of ADA varies considerably in the different studies and this makes results lowly reliable for some clinicians(10,47,55,64,68–89) (*Table 1*). This observation can be explained by the differences between the groups of patients, as well as the differences in concurrent medications, timing of sampling, duration of follow-up, drug dosing and methods. Furthermore, the choice of the assay used for ADA detection between the radioimmunoassay (RIA), enzyme-linked immosorbent assay (ELISA), homogeneus mobility shift assay (HMSA) or reporter gene assay (RGA) or pH-shift-anti-Idiotype Antigen binding test (PIA) greatly influences the results(61,63,64,90–92).

Association between drug and ADA levels with the clinical response

Several publications have emerged in the last years regarding the association between serum trough drug and ADA levels with the clinical response in rheumatic patients. Many of them have demonstrated that lower serum trough levels and ADA detection are associated with a poorer clinical response (47,68–73,76,93,83,94).

Fifty one consecutive RA patients treated with Ifx with a follow-up of 1 year were selected to study ADA status and its correlation with clinical outcomes(93). Antibodies against Ifx were detected in 22 patients (43%). Patients without detectable ATI (n = 29 [57%]) were significantly more often classified as responders (20 of 29 [69%]) compared to patients with detectable ATI (8 of 22 [36%]; $p = 0.04$)(93).

In a long term study (after 3 years) conducted with 272 RA patients treated with Ada, 28% of patients were ADA positive (ADA+)(47). The ADA negative (ADA-) group of patients had much higher serum trough Ada concentrations (median, 12 mg/L; IQR, 9-16 mg/L) compared to patients with antibody titers from 13 to 100 AU/mL (median, 5 mg/L; IQR, 3-9 mg/L) and also those higher than 100 AU/mL (median, 0 mg/L; IQR, 0-3 mg/L)(47). ADA+ patients more often discontinued participation due to treatment failure (38%) compared with ADA- ones (14%). Ninety-five of 196 ADA- patients (48%) had minimal disease activity versus 10 of 76 ADA+ patients (13%). Only 3 of 76 ADA+ patients (4%) achieved sustained remission compared to 67 of 196 (34%) ADA- ones(47).

In a study regarding a cohort of 292 RA patients treated with Etn, it was shown that Etn levels were significantly higher in good responders (median (IQR) 3.78 (2.53–5.17)) compared to both moderate 3.10 (2.12–4.47) and EULAR non-responders 2.80 (1.27– 3.93) (all $p<0.05$) after 6 months of therapy(95). When patients were categorized into quartiles according to the highest of Etn levels, the lowest quartile (Etn levels <2.1 mg/l) comprised 40% of all non-responders. The highest quartile (Etn levels >4.7 mg/l) comprised 35% of all good EULAR responders. Anti-Etn antibodies were detected in none of the sera(95).

De Vries *et al*. observed in a group of 38 SpA under Ifx therapy that serum trough Ifx levels were significantly ($p= 0.0001$) lower in ADA+ patients (mean: 0.02 mg/l) than in ADA- patients (mean: 12.7mg/l). Non responders by the Assessment in Ankylosing Spondylitis response criteria (ASAS-20) were more frequently in ADA+ patients ($p=0.04$) (68).

The correlation between immunogenicity and clinical response was also studied in an observational study with 115 SpA treated with Ada during 24 weeks(76). At the end of the study, 49 (43%) patients were Bath Ankylosing Spondylitis Disease Activity Index-50 (BASDAI50) responders and mean (SD) of Ankylosing Spondylitis Disease Activity Score (ASDAS) for responders was 1.5 (1.0) vs 2.6 (1.0) for non-responders ($p<0.001$)(76). Thirty-one (27.0%) patients had detectable ADA. After 24 weeks, serum trough levels (mg/L) (IQR) were significantly higher in ADA- patients than in ADA+ patients (12.7 (8.2–18.0) vs 1.2 (0.0–2.0), ($p<0.001$)). A significant association was demonstrated between serum trough Ada levels and ASDAS ($p=0.02$; RC −1.1; 95% CI −2.0 to −0.2). Eleven (9.6%) patients had no detectable Ada levels and high detectable ADA titres (>100 AU/mL). In these patients, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) remained high during treatment(76).

In the present study, we have studied the association between serum trough Ifx and antibodies to Ifx (ATI) levels and long-term clinical outcomes (more than 4 years) in two Spanish cohorts of RA and SpA patients(73,96). Our group was the first to show this correlation during such a broad follow up time. This association has also been analyzed with surrogate markers of clinical response, as well as the treatment survival.

Table 1. Frequency of ADA assayed by ELISA or RIA in patients with RA, PsA and SpA

Assay	Disease	Dose	ADA	Frequency (%)	Time of study (w)	Patients (n)	Reference
Infliximab							
ELISA	RA	3mg/kg	ATI	33	400	85	Pascual-Salcedo et al.,2011(96)
	RA	3 mg/kg	ATI	41.2	>360	17	Ducourau et al., 2011(71)
	RA	1,3, 10 mg/kg	ATI	17.4	26	87	Maini et al., 1998(5)
	SpA	5 mg/kg	ATI	25.5	400	94	Plasencia et al., 2012(73)
RIA	RA	3 mg/kg	ATI	43	54	51	Wolbink et al., 2006(93)
	RA	3 mg/kg	ATI	13 30 44	2 13 26	106	Bendtzen et al., 2008
	RA	---	ATI	22.2	14	18	Van den Bemt et al., 2008(97)
Fluid-phase Solid-phase Both	RA	3 mg/kg	ATI	18 12 7	12	106	Svenson et al., 2007(94)
	RA	3 mg/kg	ATI	40 50	13 26	35	Radstake et al., 2009(77)
	RA	3 mg/kg	ATI	35	54	40	Hoshino et al., 2012(80)
	AS	5 mg/kg	ATI	29	54	38	De Vries et al., 2007(68)
	SpA	5 mg/kg	ATI	15.3	156	91	Ducourau et al., 2011(71)

Assay	Disease	Dose	ADA	Frequency (%)	Time of study (w)	Patients (n)	Reference
Adalimumab							
ELISA	RA	40 mg bi-w	ATA	4.9	56	30	Van der Bijl et al., 2008(98)
	RA	40 mg bi-w	ATA	12	26	434	Van de Putte et al., 2004(99)
	RA	40 mg bi-w	ATA	7	---	57	Rosas et al., 2014(100)
RIA	RA	40 mg bi-w	ATA	47.2	52	36	Chen et al., 2015(74)
	RA	40 mg bi-w	ATA	28	156	272	Bartelds et al.,2011(86)
	RA	40 mg bi-w	ATA	17	28	121	Bartelds et al., 2007(69)
	RA	40 mg bi-w	ATA	25 30	12 26	34	Radstake et al., 2009(77)
	RA	40 mg bi-w	ATA	20	28	235	Bartelds et al., 2010(87)
	PsA	40 mg bi-w	ATA	22	52	103	Vogelzang et al., 2014(70)
	AS	40 mg/1-3 w	ATA	11.3	24	115	Kneepkens et al., 2013(76)
Etanercept							
ELISA	RA	50 mg/w	ATE	0	26	292	Jamnitski et al., 2012(95)
	RA	50 mg/w	ATE	3	16	420	Keystone et al., 2004(10)
	RA	50 mg/w	ATE	5	28	214	Dore et al., 2007(101)
	RA	25 mg x 2 w	ATE	0	32	40	Hoshino et al,2012(80)
RIA	AS	25 mg x 2 w	ATE	0	26	53	De Vries et al., 2009(10)
	RA	50 mg/w	ATE	0	52	34	Chen et al., 2015(74)

Assay	Disease	Dose	ADA	Frequency (%)	Time of study (w)	Patients (n)	Reference
Golimumab							
ELISA	RA	50 mg/m	ATG	15.2	24	33	Chen et al., 2015(89)
	SpA	50 mg/m	ATG	2.3	24	43	Chen et al, 2015(89)
RIA	RA	50 mg/m	ATG	8.1	24	37	Kneepkens et al., 2014(102)
Certolizumab							
RIA	RA	400 mg/4w	ATC	37	52	115	Jani et al.2017(83)
Tocilizumab							
ELISA	RA	---	ATT	0.8	52	126	Benucci et al., 2016(84)
	RA	---	ATT	3.2	---	40	Sigaux et al., 2016(82)
	RA	---	ATT	1.2 (sc) 0.75 (iv)	--- ---	3099 5875	Burmester et al., 2016(81)
RIA	RA	8 mg/4w	ATT	1.5	24	66	Kneepkens et al.,2016(85)

w=week; bi-w=biweekly; m=monthly; ATI=antibodies to Ifx, ATA= antibodies to Ada; ATE= antibodies to Etn;

ATG= antibodies to Goli; ATC= antibodies to Certo; ATT= antibodies to Tcz

Another very new aspect investigated in this work is the reason to why not all ATI+ patients have a bad clinical course(103). When evidence is reviewed, some ATI+ patients present a moderate clinical response and it is not clear which differences in the subgroup of ATI+ patients may be the reason for this clinical variability. This question is partially answered in one of our articles.

Association between immunogenicity and concomitant therapy with methotrexate (MTX)

Maini *et al*. first investigated whether MTX could reduce the immunogenicity of Ifx in RA patients. In this multicenter trial, 101 RA patients were randomized into seven groups of 10-15 patients each, given alone or in combination with MTX, with different Ifx dosing regimen. The detection of ATI was inversely associated with Ifx dose (53%, 21% and 7% in patients receiving 1, 3, 10 mg/kg monotherapy, respectively) and the concomitant use of 7.5 mg weekly of MTX greatly decreased the ATI detection(5).

In a study of RA patients treated with Ifx it was shown that after 6 months of treatment, ADA+ patients receiving MTX had lower ADA levels than those not receiving MTX(104). Concomitant use of other DMARDs such as sulphasalazine (SSZ), ciclosporin, hydroxycloroquine (HDQ) or prednisolone did not significantly affect ADA levels(104).

Similar findings have been confirmed in RA patients treated with Ada. In a propective RA cohort under Ada treatment over 28 weeks, the concomitant use of MTX was related to a lower rate of antibody development than patients with Ada under monotherapy (12% vs. 38%) (78).

The relationship between immunogenicity and MTX was further explored by Krieckaert *et al.* in the same group of patients, who demonstrated a clear dose-dependent relationship with MTX and the proportion of ADA+ patients(105). RA patients (n=272) under Ada treatment were stratified according to the baseline MTX dose: no concomitant MTX, low dose (5-10 mg weekly), intermediate dose (12.5-20 mg weekly) or high dose (≥22.5 mg weekly)(50). In general, patients using MTX less often

developed ADA compared to patients who were under monotherapy with TNFi (OR: 0.20, 95% CI: 0.12-0.34; *p=0.001*). The proportion of ADA+ patients inversely correlated with the MTX dose(50). The group with high dose of MTX contained the lowest proportion of patients developing immunogenicity(50) *(Figure 6)*.

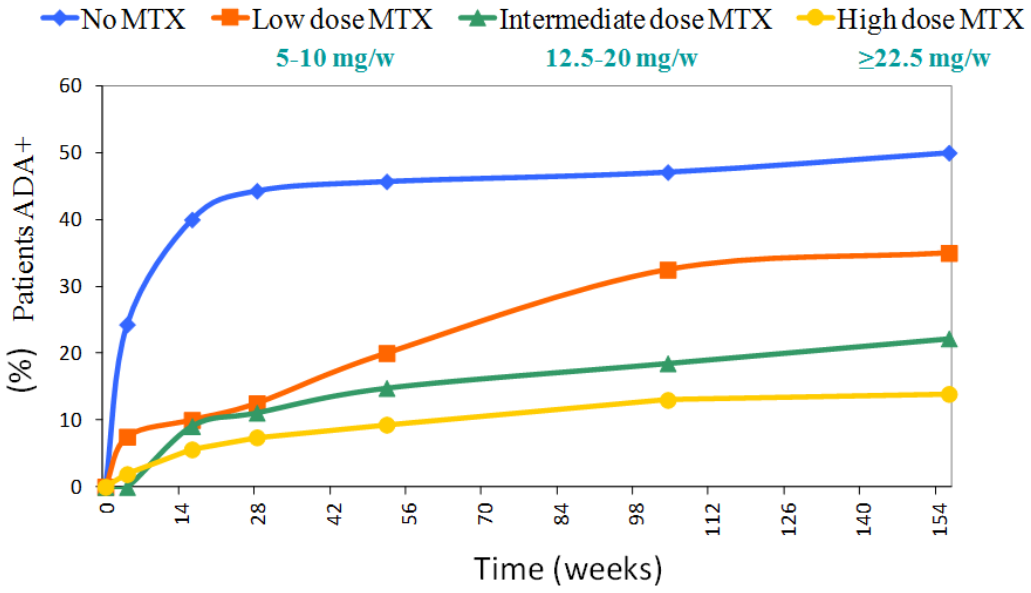

Figure 6. Effect of MTX on the Adalimumab immunogenicty. Adapted from Krieckaert et al. "*Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner*". Ann Rheum Dis 2012 Nov;71 (11):1914-5

The use of MTX has also been associated with a lower incidence of ADA to Goli in PsA (106) and AS(107) in the context of clinical trials. The GO-REVEAL trial of Goli in PsA reported a low incidence of ADA (4.6% at 6 months), however, these were present in none patients taking concomitant MTX. The efficacy of MTX use in conjunction with Ifx has previously been evaluated in AS outside the context of immunogenicity with conflicting results(48,108). An open label study with 19 AS patients assessed whether the addition of MTX could increase the therapeutic efficacy at 30 weeks(109). The 9 patients who were on low dose of MTX (7.5 mg weekly) achieved a significant better BASDAI50 compared with patients under monotherapy(109). Nevertheless, it should be taken into account that patients with combination treatment were younger and had shorter disease duration(109).

An interesting recent study conducted in PsA (n=103) and RA patients (n=272) evaluated the influence of MTX and other DMARDs on the serum Ada concentration(46). Authors concluded that Ada trough concentrations are the highest in patients taking MTX, with or without other DMARDs; and that patients under Ada monotherapy have the lowest concentrations(46). Although MTX has the strongest influence on Ada trough concentrations in this study, other DMARDs also seem to have a beneficial effect on drug levels(46).

In relation to this issue we provide information on the relevance of concomitant MTX use with TNFi like Ifx in a Spanish cohort of RA and SpA patients(73,96). A possible interpretation would be that the combination strategy not only influences ATI levels, but also drug survival(73,96).

Association between immunogenicity and adverse events

Patients who develop antibodies to biologics are more likely to show infusion-related reactions(72,73,104,110–113). Acute infusion reactions, including anaphylaxis, develop in a close temporal relationship to an infusion(110,111). The acute reactions can be truly allergic, namely IgE-mediated type I reactions, including hypotension, bronchospasm, laryngeal or pharyngeal edema, wheezing and/or urticaria. In patients with Crohn's disease an increased risk of infusion reactions was observed in patients with higher ATI levels (49,114). In a small study using radio labeled Ifx it was demonstrated that in addition to the quantity of ATI, the quality of the response is related to infusion reactions(49). Many of the ATI are of the IgG4 and IgG1 isotype(49,115). IgG4 antibodies are considered to be less inflammatory as they do not activate the complement system. In a study of 19 patients with infusion reactions to Ifx an association with the level of ATI was indeed observed. However, no protective effect of specific IgG4 was found (49). In patients who receive biological subcutaneously local injection reactions are frequently seen; the relation to antibody formation is, however, unclear. In some patients treated subcutaneously with biologics a systemic response is observed(116), but little information is available on the clinical effects of chronic immune-complex formation in these patients. In patients receiving Rtx,

immunogenicity has been linked to a delayed type of hypersensivity reaction with purpura that mimics a vasculitis-like syndrome(117).

Ducourau et al. observed in a study in RA and SpA patients under Ifx that 52% (n=11) of ATI+ patients developed at least one infusion-related reaction in comparison with only 1 (1%) ATI-patients(71). The median interval between ATI detection and reactions was 42 days. These infusion reactions include rashes, hyperthermia, chills, Quincke´s oiedema and tachycardia. Among the 11 ATI+ patients who had an infusion related reaction, 4 required intra-venous corticosteroids and antihistamine, and 2 required only per oral antihistamine. One patient developed Guillain Barré syndrome, which partially improved after polyvalent immunoglobulin treatment. In 4 cases, no treatment was required(71).

Few detailed publications are available to explain the relationship between infusions related reactions, ADA development and TNFi in rheumatic patients. In the presented study, we have published our experience about infusion related reaction in our patients treated with Ifx in the Biologic Unit of the La Paz University Hospital. Besides, information about ATI levels and the frequency of infusion related reactions is provided, as well as its correlation with MTX taking(96,118).

Clinical repercussion of early drug levels monitoring

Since 2006, Bendtzen et al. have found that low Ifx levels at 1.5 months predicted antibody development and later treatment failure in a cohort of 106 RA patients under Ifx therapy(104). In addition, they concluded that high baseline disease activity, judged by CRP levels and Disease Activity Score-28 (DAS28), related to low Ifx levels at early stages of therapy and subsequent development of ATI(104).

In a prospective cohort of 331 RA patients under TNFi (160 treated with Ada and 171 with Etn), ADA were detected in 25% of patients under Ada treatment and in none patients under Etn treatment. At 3 months, ADA formation and low Ada levels were significant predictors of no EULAR response at 12 months(44). Similar findings were shown from a French group in a pilot study with a reduced number of patients

treated with Etn. They observed that Etn concentrations at 3 months predict response to Etn therapy at 6 months, but no data about survival of the drug were provided(119). Low Etn concentrations could explain low levels of response as suggested previously(119).

In relation to Ifx at early stages, Ducourau et al. have shown in a RA and SpA cohort under Ifx that trough Ifx concentration during treatment initiation (weeks 2 and 14) was lower for ATI+ than ATI- patients and the difference was as early as week 2(71) (*Figure 7*). Another group has also described the association of early drug levels and clinical activity to predict clinical outcomes. Kobayashi et al. observed in a cohort of ulcerative colitis (UC) that early Ifx levels at week 2 in combination with clinical evaluation, is useful to predict short-long-term outcomes(120).There is sparse evidence available about biologic drug levels at early stages in rheumatic diseases. A Dutch group showed in a 57 RA patients treated with Ifx that the combination of DAS28 at week 6 of therapy with Ifx trough levels could be predictors of clinical response at 6 months(121).

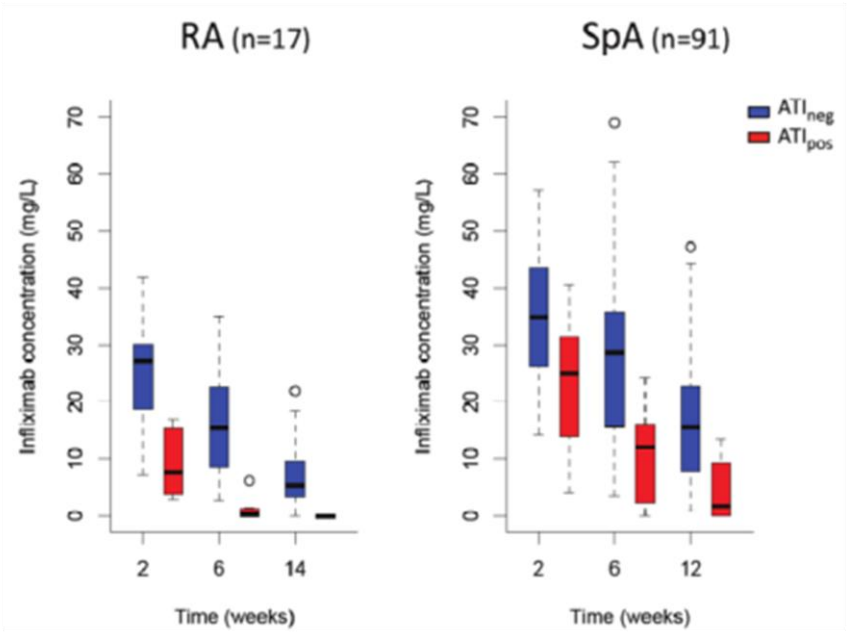

Figure 7. Infliximab concentrations at initiation in ATI positive (ATI pos) and ATI negative (ATI neg) patients. Adapted from Ducourau et al. *"Antibodies toward infliximab are associated with low infliximab concentration at treatment initiation and poor infliximab maintenance in rheumatic diseases"*. Arthritis Research & Therapy 2011, 13:R105

In our cohort of RA patients, we have obtained very novel and interesting data on this topic. Firstly, the early Ifx levels (at weeks 2, 6 and 14) have been correlated with clinical outcomes during the first year of therapy (article 4). Furthermore, we have defined a serum level cut-off at early stages of treatment to predict ATI status and clinical response during the first year under Ifx therapy (article 4). Moreover, this study has demonstrated that the differences in drug levels at early stages are partly conditioned by an early ATI production detected with immune-complex dissociation assays (article 4).

Therapeutic drug monitoring (TDM) strategies

In the absence of response to a first TNFi, different strategies, some of them proposing drug levels as the main predictor of response are routinely used, such as a change in the current TNF inhibitor dose, shortening infusion intervals (notably for Ifx), switching to another TNFi or switching to a drug with a different mechanism of action(122). There is at present no accepted rationale for the approach to primary and secondary failure of TNFi, and this means an important issue from both a clinical and economic perspective. The Dutch group has proposed that measurement of ADA before switching may be of value in clinical decision making(87,123). In RA, anti-TNF naive patients and switchers with ADA against their first TNFi showed similar responses to a second TNFi which were superior to the responses of switchers without detectable ADA(87,123) This suggests that TNFi failures in the absence of ADA may have a target-related reason for anti-TNF failure, and thus be better suited to a drug with a different mechanism of action, whilst those with ADA may have a drug-related failure.

For many biologics, mainly Ifx, increasing the dose has been proposed in patients with primary or secondary failure(124–126). Some studies have shown that therapeutic intensification significantly improves responses(124), whereas other showed little or no effect(125,126). Most of these studies did not monitor serum drug concentration or ADA. It is important to define if this intensification therapy regimen is effective in order to prevent toxicity and high costs. We know that the answer to these

questions will be provided with a prospective study but we will report our preliminary data in an observational cohort and these results can help to run a clinical trial.

Many algorithms have been proposed to implement the use of serum trough drug and ADA levels in clinical practise(18,127–130) (*Figure 8*). Most of these algorithms combine clinical outcomes with drug and/or ADA status. Garcés et al. proposed a new algorithm considering ADA status and clinical response and compared the concordance of this algorithm with the current clinical practise comparing the effectiveness of "immunogenicity-based" versus "empirical-based" switches in a cohort of RA patients receiving biologics(127). Finally, it was observed that the "immunogenicity-based" group had more probability to achieve clinical response than the other group(127). Currently, more studies are emerging to reinforce the incorporation of drug/ADA levels monitoring as a tool in the clinical evaluation of the rheumatic patients. This process is known as TDM.

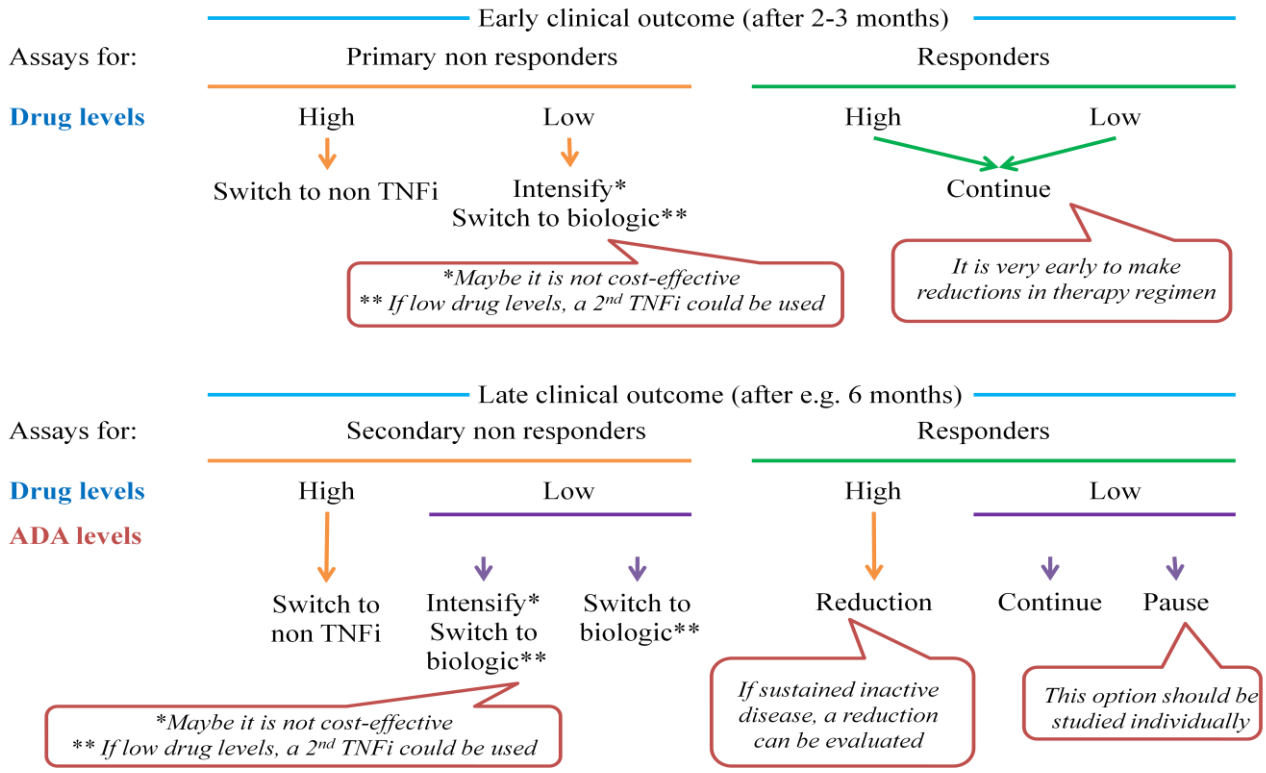

Figure 8. Algorithm to make therapeutic decisions using drug/ADA levels monitoring . Adapted from Bendtzen et al. *"Is There a Need for Immunopharmacologic Guidance of Anti–Tumor Necrosis Factor Therapies?"*. Arthritis and Rheumatism 2011 April;63 (4):867-70

In the last year, it is becoming more common to reduce the dose and/or increase the administration interval of biologics (tapering strategies) in patients with sustained low disease activity or remission. This attitude is for purposes of avoiding overtreatment in patients with long-standing exposition to biologics, decreasing the risk of infections or adverse events and using cost-effective strategies. Although there is increasing evidence that the tapering strategy is feasible in some patients clinicians are concerned about long-term clinical or structural consequences(125,131–140).

In the present work, we will provide an exhaustive review about the current situation regarding tapering strategies or discontinuation of biologics in axial SpA patients. Moreover, two interesting studies comparing long-term clinical outcomes between patients under a biologic tapering strategy versus patients with standard therapy regimen will be presented. For these studies, short and long-term clinical outcomes were collected, including the incidence and number of flares in both populations: the tapering group and the control group (with standard dose regimen). Besides, data demonstrating that TDM use in our cohort of rheumatic patients under biologics is a cost-effective strategy will be shown.

HYPOTHESIS

Our hypothesis were:

I. A faster TNFi clearance and ADA formation are associated with a poorer clinical response in rheumatic patients.

II. The concomitant therapy with immunosuppressive as MTX has an influence on the serum trough drug and ADA levels in rheumatic patients under TNFi.

III. Patients under Ifx therapy who develop ADA present lower Ifx levels since the induction phase of treatment.

IV. The development of ADA against to the first TNFi may determine the clinical response to a second TNFi.

V. Increasing dose of biological drugs is not an effective long-term therapeutic strategy

VI. A biologic tapering strategy is feasible in rheumatic patients in low disease activity or remission with a good control of the disease and important reduction of the administered drug.

VII. The economic impact of the biological therapy optimization in patients with chronic inflammatory diseases by monitoring the drug and ADA levels is remarkable.

OBJECTIVES

The main objectives of this study are:

I. To analyze the clinical relevance of the detection of ADA in RA and SpA patients under biological drugs. Several aspects will be evaluated: effect of concomitant MTX use, incidence of adverse event, timing of ADA appearance, influencing on the switching to a second TNFi.

II. To analyze whether patients who develop ADA present differences on serum drug levels since the induction phase.

III. To analyze the relationship between serum drug levels and the clinical response to the treatment with biological drugs. This will be done with different biopharmaceuticals as Ifx, Tcz, and Goli.

IV. To analyze the effect of the TDM on the treatment: effect of drug increase, TNFi discontinuation, tapering in patients with low disease activity or remission, consequences on cost-effectiveness.

PUBLICATIONS

Global Summary

Chapter 1:

Association between drug and ADA levels with clinical outcomes

ARTICLE 1: ***"Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis"*** (72)

In this study 85 RA patients from RA-Paz cohort under Ifx treatment were included. Clinical activity, response and serum trough Ifx and ATI levels were measured at baseline, 6 months, 1 year and more than 4 years of treatment.

ATI were detected in 28 (33%) patients along the study. At baseline no significant differences in clinical activity between ATI+ versus ATI- patients were found. ATI+ patients showed a higher clinical activity measured by DAS28 at all studied point (6 months: 4.85±1.24 ATI+ vs 3.67±1.24 ATI-, p=0.004; 1 year: 4.95±1.24 ATI+ vs 3.13±1.17 ATI-, p=0.004; >4 years: 4.00±1.35 ATI+ vs 3.46±1.22 ATI-, p=0.004). At the end of the study, 100% of EULAR non responders were ATI+ patients and only the 24% of EULAR (good and moderate) responders were ATI+.

Ifx trough levels were higher in EULAR responders; however, ATI levels were significantly higher in EULAR non responders at all studied point (*Figure 9*).

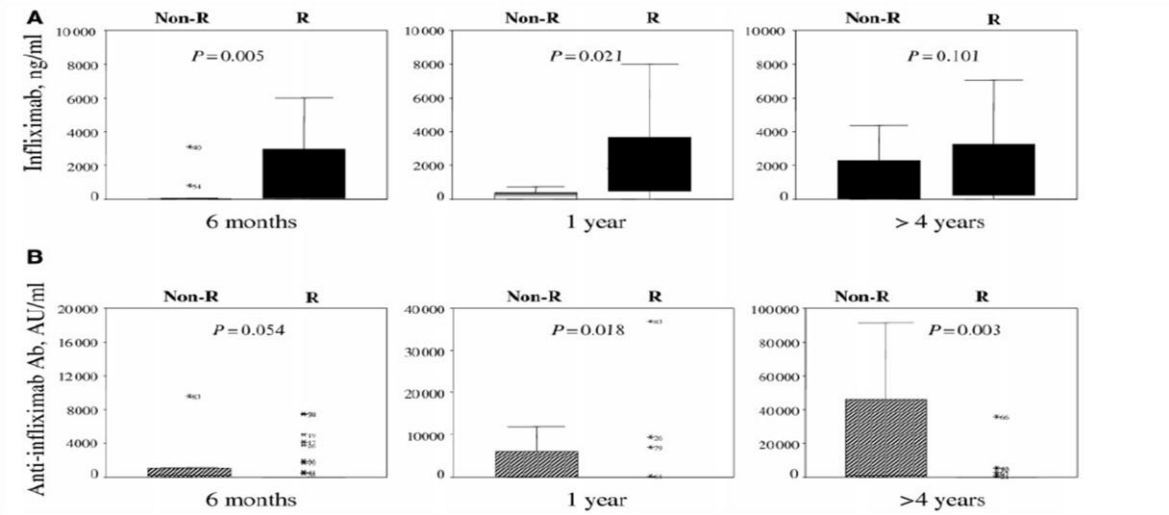

Figure 9. Serum Ifx and ATI concentrations at different studied points. Pascual et al. *"Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis"*. Rheumatology 2011;50:14451452

Forty five (54%) patients discontinued Ifx therapy and this proportion was higher in ATI+ patients (23/28 (82.1%) ATI+ patients vs 22/57 (39%) ATI- patients, p<0.001). Among ATI+ patients, Ifx survival was longer in patients taking concomitant therapy (p=0.022) (*Figure 10*).

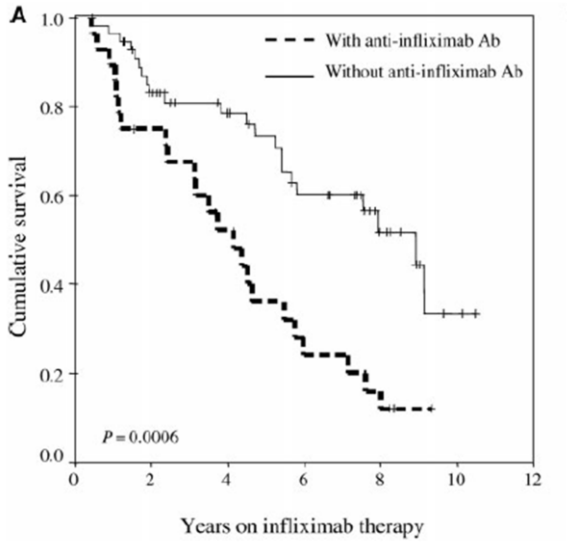

Figure 10. Survival of Ifx therapy in relation to ATI status. Pascual et al. *"Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis"*. Rheumatology 2011;50:14451452

Infusion related reactions appeared in 9 patients, all of them with detectable ATI. Among the 28 ATI+ patients, ATI levels were higher in the group with infusion related reactions (*Figure 11*).

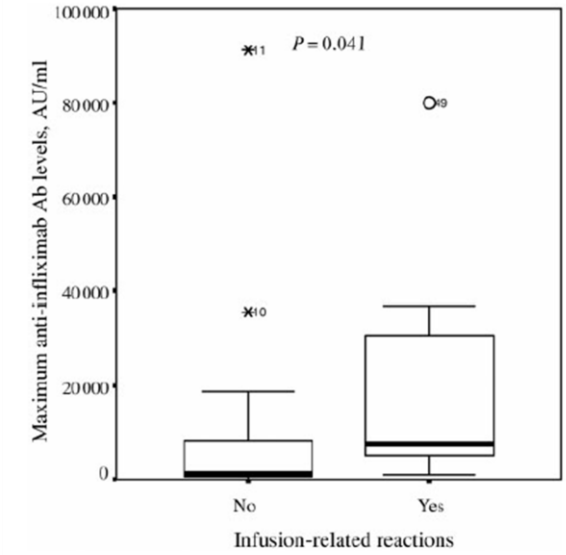

Figure 11. Comparison of ATI levels in patients who develop or not an infusion related reactions. Pascual et al. *"Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis"*. Rheumatology 2011;50:14451452

We did not find a lower proportion of patients developing ATI in association with MTX use (32% with MTX vs 37% without MTX, p=0.77). However, in ATI+ patients receiving MTX, maximal levels (Mdn-IQR) tended to be lower than in those ATI+ patients without MTX (3414, 808-7426 AU/ml with MTX vs 21250, 7049-47656 AU/ml without MTX, p=0.07).

ARTICLE 2: *"Influence of immunogenicity on the efficacy of long-term treatment of spondyloarthritis with infliximab"* (73)

Ninety four axial SpA from SpA-Paz cohort treated with Ifx were included in this study. Clinical activity and serological parameters were measured at baseline, 6 months, 1 year and >4 years of treatment.

ATI were detected in 24 (26%) patients, in 71% of them within the first year of therapy. ATI+ patients had a more active disease by ASDAS than ATI- patients at all studied time point ((6 months: 2.55±0.89 ATI+ vs 1.79±1.04 ATI-, p=0.038; 1 year: 1.95±0.67 ATI+ vs 1.67±0.71 ATI-, p=0.042; >4 years: 2.52±0.99 ATI+ vs 1.53±0.81 ATI-, p=0.004). During the study, 51 (54%) patients had a clinically significant improvement and only 9 of them were ATI+ (p=0.047). Ifx trough levels were higher in inactive patients at any studied point (*Figure 12*).

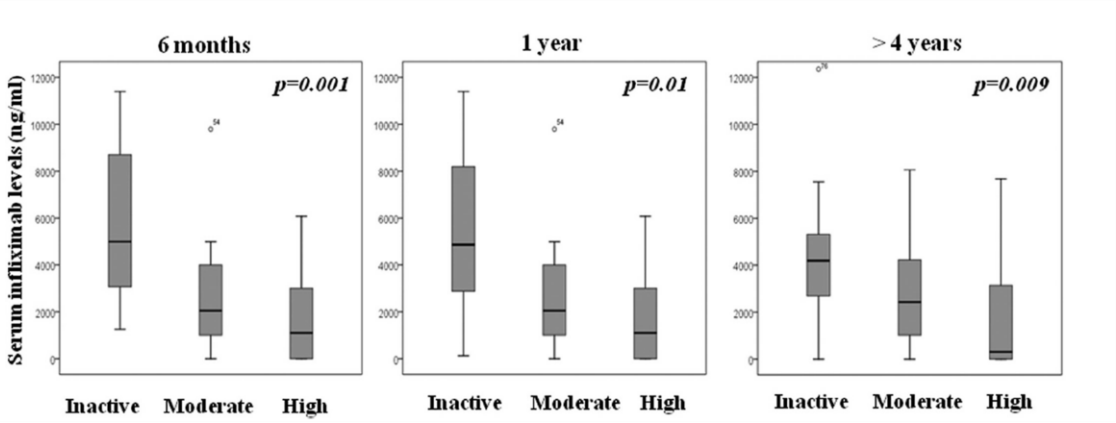

Figure 12. Association between serum Ifx concentrations with clinical activity by ASDAS in SpA patients. Plasencia et al. *"Influence of immunogenicity on the efficacy of longterm treatment of spondyloarthritis with infliximab".* Ann Rheum Dis 2012;71:1955–1960

Twenty seven (29%) patients dropped out Ifx during the study, most of them ATI+ patients (18/24 (75%) ATI+ versus 9/70 (13%) ATI-, p>0.001).

Infusion related reactions were seen in 11 SpA patients, the majority of them in the ATI+ patients (73% ATI+ vs 27% ATI-, p=0.001). Moreover, ATI levels were higher in the patients who developed an infusion related reaction (p=0.028) (*Figure 13*).

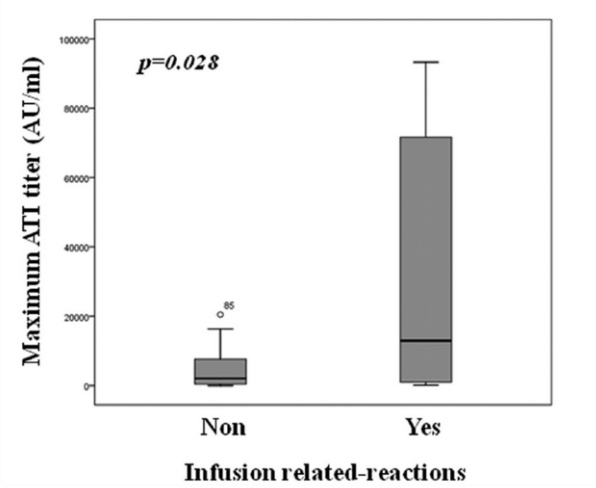

Figure 13. Comparison of ATI levels in patients who develop or not an infusion related reactions. Plasencia et al. *"Influence of immunogenicity on the efficacy of longterm treatment of spondyloarthritis with infliximab"*. Ann Rheum Dis 2012;71:1955–1960

ATI were detected more frequently in patients not taking MTX than in patients with concomitant MTX (20/58 (35%) without MTX versus 4/36 (11%) with MTX, p=0.011). Moreover, the maximum Ifx levels (mean ±SD) tended to be higher in patients taking concomitant MTX than patients with biological drug in monotherapy (4548 ±3832 with MTX vs 3485 ±3018 without MTX, p=0.147).

ARTICLE 3: *"The timing of serum infliximab lose, or the appearance of antibodies to infliximab (ATI) is related with the clinical activity in ATI-positive patients with rheumatoid arthritis"* (103)

To understand the reason why not all ATI+ patients have a poor clinical response, we investigated whether the timing of ATI appearance and/or drug disappearance is correlated with clinical activity, measuring the Ifx and ATI levels 4 weeks after infusion (n+4).

Eleven ATI positive RA patients were included in this study. Patients who had detectable ATI four weeks earlier (half cycle) showed higher disease activity measured by DAS28 (6.56±0.69 ATI+ at half cycle vs 4.56±0.93 ATI- at half cycle, p=0.012) and worse clinical improvement measured by delta-DAS28 (-1.22±0.72 ATI+ at half cycle vs 0.94±0.93 ATI- at half cycle, p=0.012) at the study point (*Figure 14*). Moreover, ATI

levels were higher in ATI+ patients at half cycle (Mdn, IQR: 8038 (2640-12063) ATI+ at half cycle vs 260 (131-342) ATI+ at half cycle p=0.024).

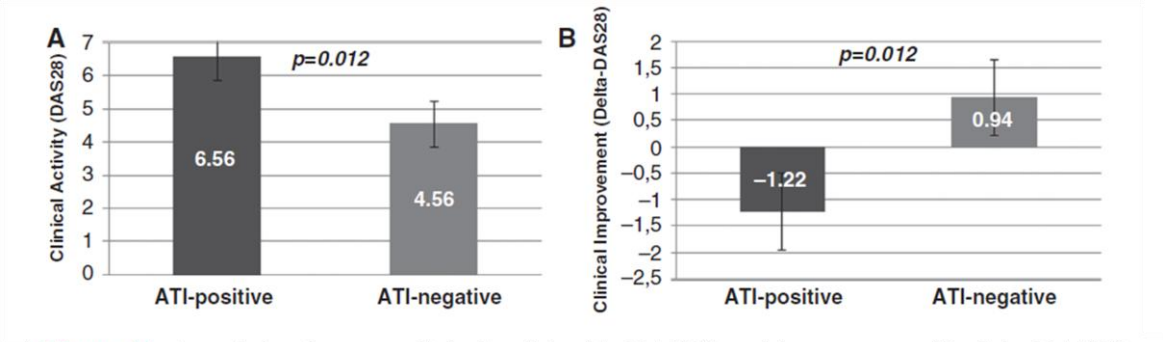

Figure 14. Association between clinical activity (A: DAS28) and improvement (B: delta-DAS28) at week n+8 with ATI status (positive or negative) at week n+4. Plasencia et al. *"The timing of serum infliximab loss, or the appearance of antibodies to infliximab (ATI), is related with the clinical activity in ATI-positive patients with rheumatoid arthritis treated with infliximab"*. Ann Rheum Dis November 2013 Vol 72 No 11

ARTICLE 4: *"Predictive Value of Serum Infliximab Levels at Induction Phase in Rheumatoid Arthritis Patients"*

This is an observational study in which Ifx trough levels (ITL) from 66 RA patients were measured by ELISA at week (W) 0, W2, W6, W14 and W22. Patients were classified as ITLpos if Ifx was detectable at W54 and ITLneg otherwise. ATI were assayed by bridging ELISA and by two drug-tolerant assays. ITL cut-off values were established by receiver operating characteristic (ROC) curves.

The ITLneg patients at W54 had lower early-stage ITL than the ITLpos patients (W2: 20.0 ±12.7 µg/mL vs 29.7±14.5 µg/mL (p=0.015); W6: 4.2±5.9 µg/mL vs 15.7±11.1 µg/mL (p<0.0001); W14: 0.1±0.2 µg/mL vs 4.1±5.3 µg/mL (p<0.0001); and W22: 0.01±0.04 µg/mL vs 2.8±3.3 µg/mL (p<0.0001)) (*Figure 15*). At all studied time points, the areas under the curve (AUC) of the ROC curves were statistically different from 0.5, enabling cut-off levels to distinguish between ITLpos and ITLneg patients at W54. The W6 predictive cut-off (4.4 µg/mL) showed the best association with IFX absence at W54, with a sensitivity of 70% (95% confidence interval (CI): 45.7-88.1), a specificity of 95% (95% CI: 83.1-99.4) and a positive likelihood ratio of 14 (*Figure 16*).

Therefore, the W6 predictive cut-off was considered the reference value to predict clinical and serological outcomes for further analysis.

In the univariable analysis, three factors were significantly associated with Ifx absence at W54: i) having an ITL below the cut-off at W2 (odds ratio (OR): 12.40; 95% CI: 3.48-44.15) or at W6 (OR: 44.33; 95% CI: 7.99-246.03), ii) non-use of MTX (OR: 4.20; 95% CI: 1.33-13.32) and iii) being older (OR: 1.04; 95% CI: 1.03-1.07).

In the multivariable logistic regression analysis, even after adjusting for possible confounders (age, gender and baseline DAS28), the ITL below the cut-off at W2 (OR: 15.85; 95% CI: 2.95-85.03; p=0.01) or at W6 (OR: 86.64; 95% CI: 6.58-1139.99) and also the non-use of MTX (OR: 12.26; 95% CI: 1.83-82.22) remained significantly associated with Ifx absence at W54.

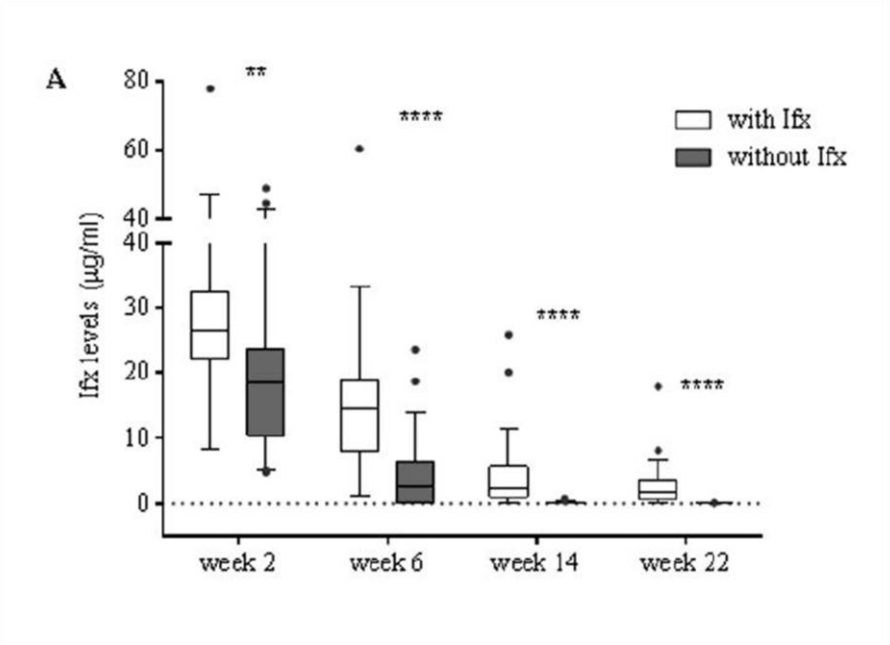

Figure 15. Serum Ifx levels at early weeks in patients with/without Ifx at 1 year of therapy. Jurado et al. *" Early-stage Infliximab serum trough levels predict drug loss levels and clinical response at one year in patients with rheumatoid arthritis".* Submitted in The Open Journal of Rheumatology.

Patients with ITL above the W6 predictive cut-off (4.4 µg/ml) had lower DAS28 scores at W54 than patients with ITL below the cut-off (3.68±1.26 vs 4.75±1.27; p=0.01). Most patients with low disease activity or remission by DAS28 at W54 had ITL above predictive cut-off at W6 (ITL above: 20 of 45 patients (44%) vs ITL below: 3 of 19 patients (16%); p=0.02).

Week	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	LR+
2	21.2 µg/mL	67% (44-84)	86% (70-95)	4.8
6	4.4 µg/mL	70% (45-88)	95% (83-99)	14
14	0.4 µg/mL	83% (35-99)	89% (75-97)	7.9
22	0.2 µg/mL	100% (15-100)	94% (81-99)	18

CI, confidence interval; LR, Likelihood Ratio.

Figure 16. The cut-off, sensitivity, specificity and likelihood ratio of serum Ifx levels at early weeks associated with Ifx disappearance during the 1st year of therapy. Jurado et al. *" Early-stage Infliximab serum trough levels predict drug loss levels and clinical response at one year in patients with rheumatoid arthritis".* Submitted in The Open Journal of Rheumatology.

ARTICLE 5: *"Serum Tocilizumab trough concentration can be used to monitor systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study"* (85)

This study included 66 RA patients (34 patients from the Netherlands and 32 patients from Spain). Clinical parameters were collected at baseline, 4, 12 and 24 weeks in the Dutch cohort and at baseline and 24 weeks of treatment in the Spanish cohort. Serum trough levels were measured at baseline, 4, 12 and 24 weeks in both cohorts.

In total, nine patients had Tcz levels <1mg/L in 2 subsequent visits. Only 1 out of the 3 patients with low Tcz levels had detectable ADA.

Tcz concentration above 1 mg/L was sufficient to normalize the serum CRP levels (≤10 mg/L). Spearman´s rank correlation coefficient showed a significant negative correlation between Tcz and CRP levels (-0.460, p<0.001).

Twelve patients (18%) did not achieve a significant improvement by delta-DAS28 at 24 weeks (delta-DAS≥1.2) and the 67% (8/12) of them had Tcz concentration <1mg/L. A linear regression coefficient of 0.080 (95% CI: 0.039-0.113, p<0.001) for the association between Tcz concentration and delta-DAS28 at 24 weeks was found.

ARTICLE 6: ***"Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice"*** (102)

This prospective observational study included 37 RA patients treated with Goli at standard therapy regiment (50 mg sc monthly), recruited from the Spanish cohort of La Paz-Spain and from the Dutch cohort of Reade Centrum-the Netherlands.

At week 52, 15 patients (41%) were responders and 22 (59%) non-responders by DAS28. The 51% (19) of patients dropped out Goli during the first year of therapy (11 for inefficacy, 7 for side effects and 1 for other reasons).

The median of Goli levels measured in serum was significantly higher in responders compared with non responders (1.36, 0.5-1.82 in responders versus 0.43, 0.23-0.84 in non-responders, p=0.023).

All patients were stratified according to Goli levels into quartiles. The lowest quartile comprised the 32% of non-responders in comparison with the higher quartile that comprised the 47% of responders (*Figure 17*).

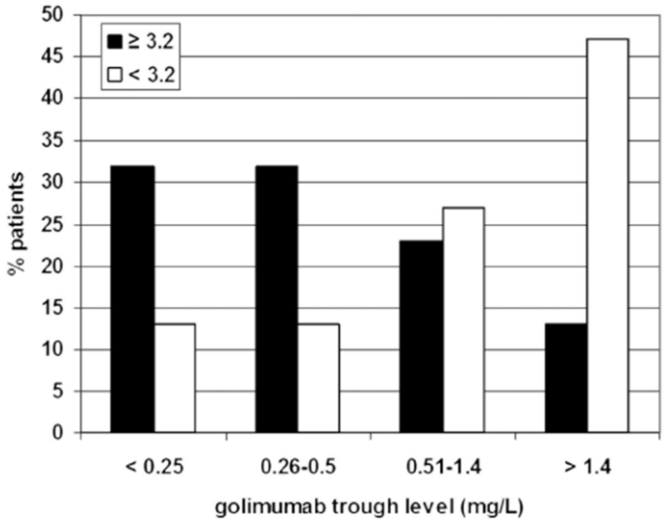

Figure 17. Association between clinical activity by DAS28 and serum Goli levels in RA patients under Goli treatment. Kneepkens et al. *"Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice"*. Ann Rheum Dis December 2014 Vol 73 No 12

Chapter 2:

Therapeutic strategies based on monitoring of drug and ADA levels

ARTICLE 7: "*The immunogenicity to the first anti-TNF therapy determines the outcome of switching to a second anti-TNF therapy in Spondyloarthritis patients*" (118)

Forty two SpA patients from SpA-Paz cohort were enrolled in this study. All 42 SpA patients received a first TNFi (20 with Ifx, 5 with Ada and 17 with Etn) and were switched to a second TNFi (9 with Ifx, 19 with Ada, 8 with Etn and 6 with Goli). Immunogenicity status against the first TNFi was analyzed before switching and clinical response to second TNFi (based on ADA pos/neg status during the 1st TNFi) was studied at 6 months of switching by ASDAS.

ADA were detected in 11 (26%) patients and appeared mainly within the first year of TNFi therapy. Most ADA negative patients against to the first TNFi had clinical inefficacy and detectable drug levels just before switching.

At 6 months after switching, ADA positive SpA patients against to the first TNFi had a lower clinical activity measured by ASDAS in comparison with ADA negative patients (1.62±0.93 ADA pos vs 2.79±1.01 ADA neg, p=0.002).

After 6 months of switching, most ADA neg against the first TNFi patients were classified as being in high or very high disease activity by ASDAS (81% ADA neg vs 27% ADA pos, p=0.002) (*Figure 18*).

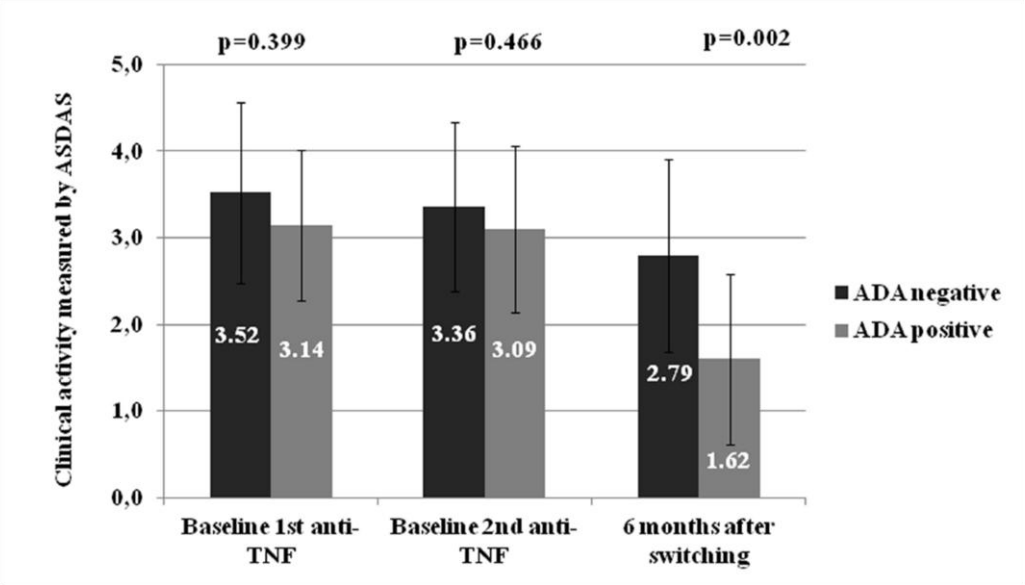

Figure 18. Comparison of clinical activity by ASDAS at different studied points between patients with/without ADA against the first TNFi. Plasencia et al. *"The immunogenicity to the first anti-TNF therapy determines the outcome of switching to a second anti-TNF therapy in spondyloarthritis patients"*. Arthritis Research & Therapy 2013, 15:R79

ARTICLE 8: ***"Effect of infliximab dose increase in rheumatoid arthritis at different trough concentrations: a cohort study in clinical practice conditions"*** (141)

A retrospective observational study, including 42 RA patients treated with Ifx from RA-Paz cohort, was carried out to analyze the effect of Ifx dose escalation strategies on clinical activity. Clinical and serological parameters were studied before dose increase (T1), 1st visit after dose increase (T2), 6 months after dose increase (T3) and 12 months after dose increase (T4). Patients were classified based on Ifx/ADA status before dose increase: group 1 with 20 without Ifx levels (ADA pos), group 2 with 13 with low Ifx levels and group 3 with 9 with high Ifx levels.

Considering the total population, DAS28 improved at T2 (4.55±1.01 at T1 vs 3.95±1.22 at T2, $p<0.005$) but this improvement disappeared at T4 (3.98±1.22 at T4, $p=0.075$). The delta-DAS28 demonstrated a significant worsening during the study (from -0.63±1.18 at T2 to 1.17±1.45 at T4, $p<0.001$).

In the analysis of individual patients groups, no significant change in DAS28 was observed throughout the study (*Figure 19*). Nevertheless, the decrease in disease

activity from T2 was significant in group 1 (1.0±1.9 at T2 vs -0.7±1.0 at T4, p<0.05) and group 3 (1.3±1.3 at T2 vs -1.0±1.3 at T4, p<0.05) after12 months (*Figure 19*).

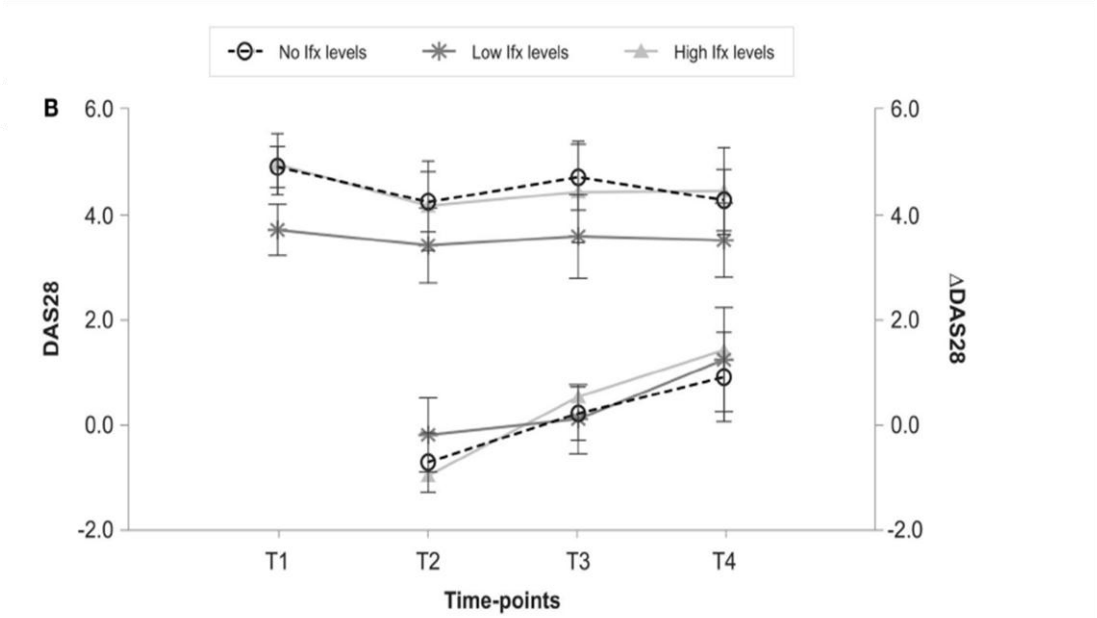

Figure 19. DAS28 and delta-DAS28 from T1 in patients with no, low and high Ifx serum concentrations at baseline. T1 (baseline), T2 (post-Ifx dose increment), T3 (at 6 months), T4 (at 12 months). Plasencia et al. *"Effect of infliximab dose increase in rheumatoid arthritis at different trough concentrations: a cohort study in clinical practice conditions "*. Frontiers in Medicine October 2015; 2 (71)

Chapter 3:

Tapering or Discontinuation strategies in rheumatic patients

ARTICLE 9: ***"Anti-TNF discontinuation and tapering strategies in patients with axial spondyloarthritis: a systemic literature review"*** (142)

Thirteen studies out of 763 retrieved citations were included. In general, published data are scarce and the level of evidence of the studies is weak. Five studies provided evidence for assessing discontinuation and eight studies evaluating tapering strategy.

On discontinuation studies, 4 of these works were observational studies after participation in RCT and 1 of them was a RCT to evaluate the effect of thalidomide to prevent flare after TNFi discontinuation. In total, they included 220 SpA patients. In these studies, patients receiving standard dose of TNFi discontinued this therapy and were followed to assess the appearance of flare. The median (range) percentage of flaring patients was 79% (76-100%). In the four observational studies, patients were retreated with the same TNFi, achieving a good clinical response in most patients.

Eight studies evaluated the tapering strategy of TNFi in SpA with LDA or remission. Dose reduction was most frequently done by increasing the interval of administration. The majority of patients maintained the LDA or reduction (proportion of patients: 53%-100%) or presented a low BASDAI (mean BASDAI: 0.6-3.2) after tapering strategies in the evaluated studies.

ARTICLE 10: ***"Comparing tapering strategy to the standard dosing regimen of TNF inhibitors in rheumatoid patients with low disease activity"*** (143)

Two groups of 144 RA patients on TNFi with DAS28<3.2 were compared: tapering group (TG: 67 patients from Spain) and control group with standard therapy regimen (CG: 77 patients from the Netherlands). The clinical and serological parameters were measured at baseline (visit 0), prior to start the tapering strategy in TG and with DAS28<3.2 in TG and CG at least 6 months (visit 1), 6 months after visit 1 (visit 2), 1 year after visit 1 (visit 3), the last visit available after visit 1 (visit 4) and the visit with the worst flare between visit 1 and visit 4 (visit-flare). In the TG, 23 patients were treated with Ifx, 23 with Ada and 21 with Etn. In the CG, 22 patients received Ifx, 27 Ada and 28 Etn.

The clinical course measured by DAS28 was similar in both groups during the study (*Figure 20*). A similar behavior was observed in the analysis between the different TNFi.

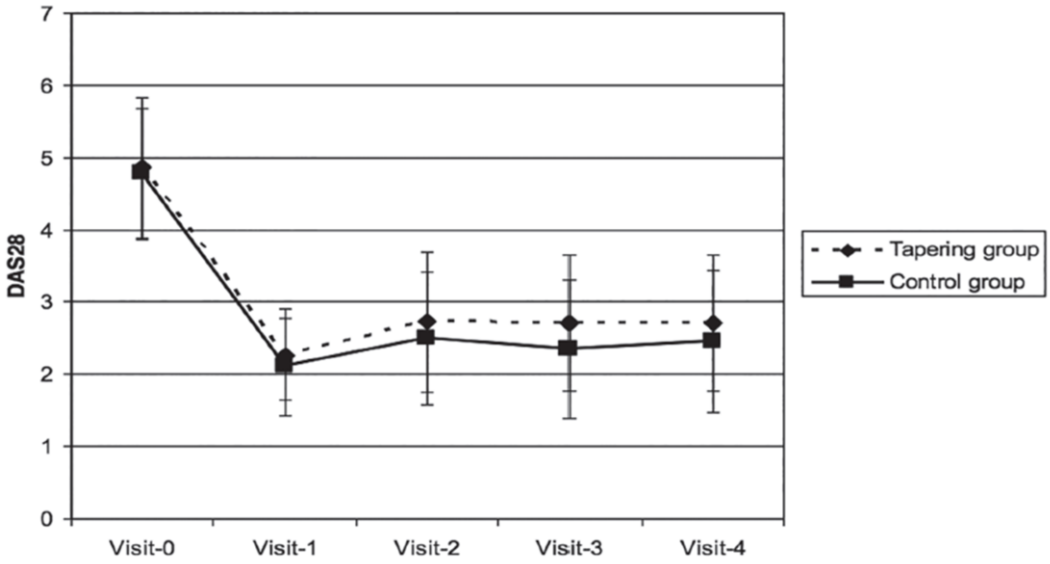

Figure 20. Comparison of clinical activity by DAS28 between tapering and control groups along the study. Plasencia et al. *"Comparing a tapering strategy to the standard dosing regimen of TNF inhibitors in rheumatoid arthritis patients with low disease activity".* Clin Exp Rheumatol. 2016 Jul-Aug;34(4):655-62

Fifty six (39%) RA patients had a flare throughout the study without significant differences between groups (39% in TG vs 39% in CG, p=0.324). Also, no differences were observed between the number of flares (1.8±0.8 in TG vs 1.7±0.7 in CG, p=0.575) or the time to appear the first flare between both groups (1.3±0.8 years in TG vs 1.4±0.7 years in CG, p=0.580).

In the TG, a significant reduction in the drug levels was observed between visit 1 and visit 4 ($p \leq 0.001$). Fourteen percent of patients were ADA positive at the end of the study and most of them were in the TG (20% ADA pos in TG vs 9% ADA pos in CG, p=0.052).

The time in low disease activity prior to start the tapering strategy (OR: 0.35; 95% CI: 0.13-0.9) was the only predictive factor that demonstrated to be protector of having a flare during the follow-up.

At the end of the study, most patients in the TG were using the therapy regiment according to the tapering strategy without requiring the use of standard labeled dose (83% in Ifx, 100 % in Ada and 86% in Etn).

At visit 4, an important reduction of administered drug was observed in TG in comparison to the CG with an interval elongation of 33% for Ifx, 53% for Ada and 53% for Etn.

ARTICLE 11: *"Comparing tapering strategy to the standard dosing regimen of TNF inhibitors in spondyloarthritis with low disease activity"* (144)

Two groups of 117 SpA patients on TNFi at least in LDA were compared: tapering group (TG: 74 patients from Spain) and control group with standard therapy regimen (CG: 43 patients from the Netherlands). The clinical and serological parameters were measured at baseline (visit 0), prior to start the tapering strategy in TG and with DAS28<3.2 in TG and CG at least 6 months (visit 1), 6 months after visit 1 (visit 2), 1 year after visit 1 (visit 3), the last visit available after visit 1 (visit 4) and the visit with the worst flare between visit 1 and visit 4 (visit-flare). In the TG, 35 patients were treated with Ifx, 17 with Ada and 22 with Etn. In the CG, 21 patients received Ada and 22 Etn. No CG for Ifx was available in the Dutch cohort.

The clinical course measured by BASDAI was similar in both groups during the study (*Figure 21*). A similar behavior was observed in the analysis between the different TNFi.

Thirty (26%) SpA patients experienced a flare along the study without significant differences between groups (30% in TG vs 19% in CG, p=0.184). Moreover, no differences were observed between the number of flares (1.4±0.7 in TG vs 1.5±0.5 in CG, p=0.486) or the time to appear the first flare between both groups (1.3±0.8 years in TG vs 1.3±1.2 years in CG, p=0.841).

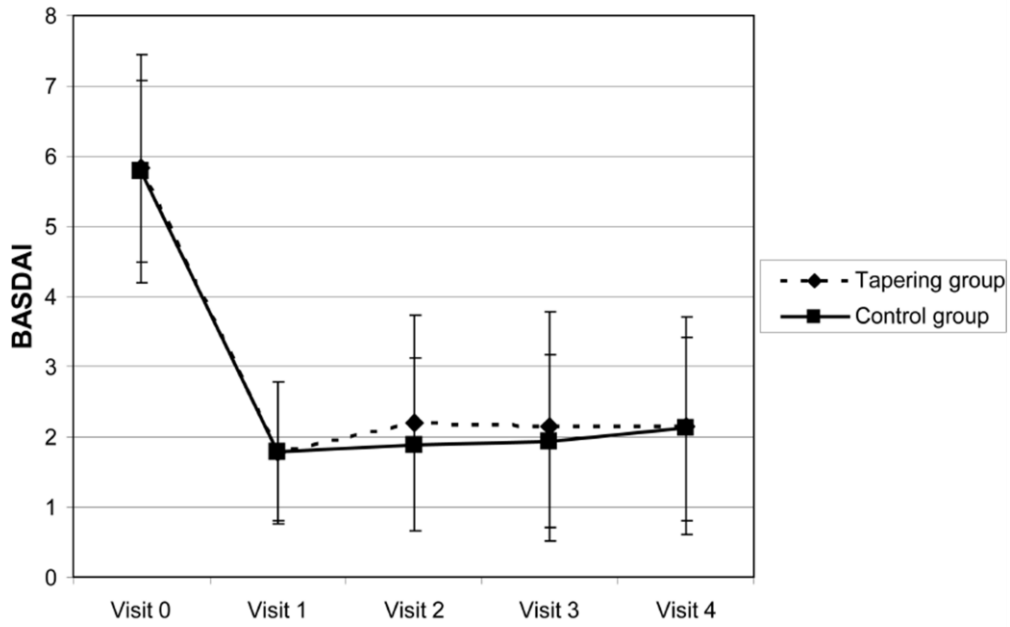

Figure 21. Comparison of clinical activity by BASDAI between tapering and control groups along the study. Plasencia et al. *"Comparing Tapering Strategy to Standard Dosing Regimen of Tumor Necrosis Factor Inhibitors in Patients with Spondyloarthritis in Low Disease Activity". The Journal of Rheumatology 2015; 42:9*

In the TG, a significant reduction in the drug levels was observed between visit 1 and visit 4 ($p \leq 0.001$). Fourteen percent of patients were ADA positive at the end of the study, being significantly more frequent in the TG (19% ADA pos in TG vs 5% ADA pos in CG, p=0.028).

To be a male (OR: 3.5; 95% CI: 1.2-10.4) was the only predictive factor that demonstrated to be protector of having a flare during the follow-up.

At the end of the study, most patients in the TG were using the therapy regiment according to the tapering strategy without requiring the use of standard labeled dose (97% in Ifx, 94 % in Ada and 86% in Etn).

Overall, the reduction of the administered drug at visit 4 in the TG was 22% for Ifx and the interval elongation was extended to 29%. The reduction of administered drug was around 45% for Ada and 52% for Etn.

Chapter 4:

Economic repercussion of tapering strategies by monitoring drug/ADA levels (TDM)

ARTICLE 12: ***"Dose-Tapering of TNF inhibitors in daily rheumatology practice enables the maintenance of clinical efficacy while improving cost-effectiveness"*** (145)

A total of 77 rheumatic patients (36 RA and 41 SpA) under TNFi with sustained LDA from RA-Paz cohort were included. The same patients were studied in two different periods: 1st period (1st P) with standard therapy regimen between 2007-09 and a 2nd period (2nd P) with a tapering strategy between 2010-12. Out of 77 RA patients, 29 were under Ifx, 27 under Ada and 21 under Etn. Clinical activity was measured by DAS28 for RA patients and by BASDAI for SpA patients.

No differences were observed in the clinical activity of patients between the 1st and 2nd P, even when a sub-analysis among the different TNFi was performed (*Figure 22*).

	DAS28			BASDAI		
	1st P	2nd P	P	1st P	2nd P	p
Ifx, n=29	2.37(0.51)	2.31(0.76)	0.78	1.72(0.72)	1.75(0.88)	0.886
Ada, n=27	2.36(0.35)	2.35(0.33)	0.908	2.10(1.44)	2.00(1.13)	0.700
Etn, n=21	2.15(0.56)	2.38(0.55)	0.124	2.07(0.81)	2.19(0.79)	0.657
Total	2.28(0.47)	2.37(0.50)	0.200	1.88(0.95)	1.90(0.93)	0.910
*mean(sd)						

Figure 22. Comparison of clinical activity by DAS28 and BASDAI in RA and SpA patients, respectively, between 1st and 2nd periods. Pascual-Salcedo et al. *"Dose-Tapering Of TNF Inhibitors in Daily Rheumatology Practice Enables the Maintenance of Clinical Efficacy While Improving Cost-Effectiveness"*. J Pharmacovigilance 2015, 3:4

In the 2nd P, the interval of drug administration was higher for all TNFi (8.7 ± 1.4 weeks in the 1st P vs 9.85 ± 1.5 weeks in the 2nd P, p<0.001 for Ifx; 2.3 ± 0.63 weeks in the 1st P vs 3.1 ± 1.02 weeks in the 2nd P, p<0.0001 for Ada; 1.4 ± 0.56 weeks in the 1st P vs 2.16 ± 1.57 weeks in the 2nd P, p<0.05 for Etn).

During the 2nd P, serum trough drug levels were significantly lower. The mean±SD serum drug levels in the 1st P versus serum drug levels in the 2nd P were 3.2 ± 2.5 μg/ml vs 1.8 ± 1.5 μg/ml (p<0.0001) for Ifx; 5.5 ± 2.8 μg/ml vs 3.1 ± 2.1 μg/ml (p<0.0001) for Ada; and 1.8 ± 1.1 μg/ml vs 1.3 ± 0.8 μg/ml (p<0.05) for Etn. The appearance of immunogenicity was not higher in the 2nd P (3 patients in the 1st P vs 7 patients in the 2nd P) and mean antibody levels in the 10 patients (17 samples) were very low (anti-Ifx antibodies: 18 ± 13 AU/ml; anti-Ada antibodies: 12 ± 8.7 AU/ml).

The amount of drug consumed decreased by 18% for Ifx, 23% for Ada and 19% for Etn in the 2nd P, resulting in an average saving of 20% in the amount of drug per year. Based on the prices of these drugs in our hospital at the end of 2012, the cost/year for each patient was significantly reduced in the 2nd P for all three TNFi (*Figure 23*); the estimated total cost saving was approximately €153,798/year, with a mean saving/patient/year of €1,715 for Ifx, €2,580 for Ada, and €1,638 for Etn.

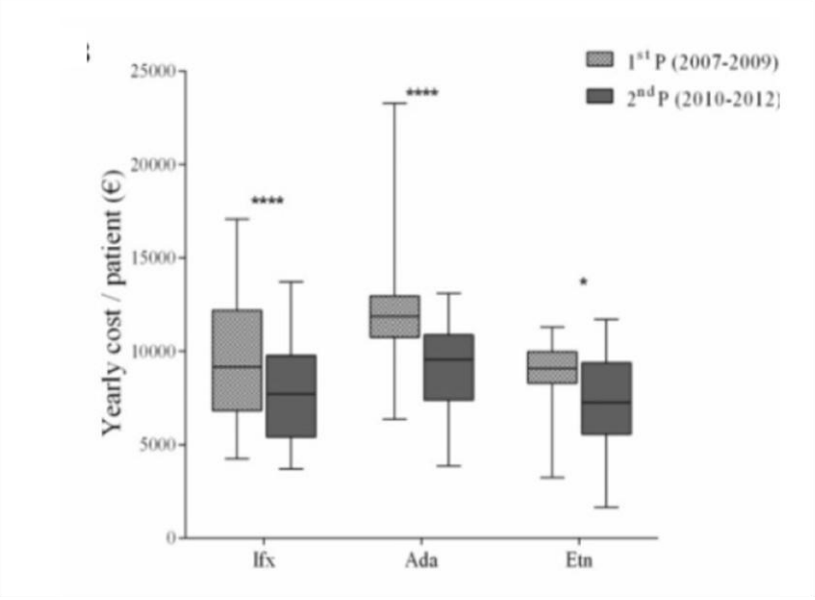

Figure 23. Annual cost per patient for each TNFi in the 1st P (2007-2009) vs the 2nd P (2010-2012), expressed in €. Pascual-Salcedo et al. *"Dose-Tapering Of TNF Inhibitors in Daily Rheumatology Practice Enables the Maintenance of Clinical Efficacy While Improving Cost-Effectiveness"*. J Pharmacovigilance 2015, 3:4

ARTICLE 13: ***"Anti-TNF dose and anti-drug antibody levels in rheumatic and psoriasis patients: economic repercussion"*** (146)

This is an ambispective observational study with a retrospective period (2009-2012) including patients without tapering strategy and a prospective period (started in 2013) including patients under a tapering strategy.

In total, the therapy regimen of biologic agents was checked in 449 adults with rheumatic diseases (209 RA and 240 SpA patients) and 167 adults with psoriasis.

Out of 209 RA patients, 145 were in subcutaneous TNFi regimen and 64 with intravenous biological agent, being 26 patients on Ifx. From 2013, the 43% of RA patients were under a tapering strategy [46.2% (67/145) under sc biologic and 36% (23/64) under iv biologic].

In the SpA group, 176 were receiving subcutaneous biologic and 64 were treated with intravenously Ifx. Beginning with 2013, 41% percent of SpA patients were under tapering strategy (39% under sc biologic and 47% under iv biologic).

Forty eight percent of psoriasis patients were under a tapering strategy from 2013 (38% under sc therapy and 44% under iv therapy).

A progressive reduction in cost/patient/year, in the departments of Rheumatology and Dermatology, was seen along the study.

Chapter 1: Association between drug and ADA levels with clinical outcomes

ARTICLE 1: ***"Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis"***

ARTICLE 2: ***"Influence of immunogenicity on the efficacy of long-term treatment of spondyloarthritis with infliximab"***

ARTICLE 3: ***"The timing of serum infliximab loss or the appearance of antibodies to infliximab (ATI) is related with the clinical activity in ATI-positive patients with rheumatoid arthritis"***

ARTICLE 4: ***"Predictive Value of Serum Infliximab Levels at Induction Phase in Rheumatoid Arthritis Patients"***

ARTICLE 5: ***"Serum Tocilizumab trough concentration can be used to monitored systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study"***

ARTICLE 6: ***"Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice"***

ARTICLE 1

TITLE: ***"Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis"***

JOURNAL: Rheumatology 2011;50(8):1445–52(72)

AUTHORS: Dora Pascual-Salcedo, **Chamaida Plasencia**, Susana Ramiro, Laura Nuño, Gema Bonilla, Daniel Nagore, Ainhoa Ruiz del Agua, Antonio Martínez, Lucien Aarden, Emilio Martín-Mola and Alejandro Balsa

PATIENTS AND METHODS:

Patients, sera and clinical assessment

A total of 85 consecutive patients with RA treated with Ifx, without previous biological treatment were included, from RA-Paz cohort. This was a retrospective observational study, approved by the Hospital La Paz Ethics Committee and patients signed an informed consent. Serum samples (a total of 1451) were collected at the time of infusion, stored frozen and only thawed for the purpose of this study. The retrospective study period covers the years 1999 until 2010. All patients fulfilled the

ACR 1987 revised criteria for RA and all of them had evidence of active disease, as indicated by a 28-joint DAS (DAS-28) at inclusion of 5.49 (1.2) [mean (S.D.)]. At first all patients were given iv infusions of 3 mg/kg Ifx at 0, 2, 6 and every 8 weeks thereafter. After 14 weeks of treatment, the rheumatologist was allowed to increase the Ifx dosage to 5 mg/kg depending on the observed clinical response. The clinical activity and response was measured by DAS28 and EULAR response criteria at baseline, 6 months, 1 year and >4 years [mean (S.D.) 5.9 (2) years]. Blood samples were collected at baseline and just before each infusion at 2, 6 and every 8 weeks thereafter, so that a maximum of 6-8 samples per year were obtained from each patient.

Serum infliximab assay

Serum Ifx levels were determined by a sandwich ELISA. Briefly, microstate plates were coated with 2 mg/ml mouse monoclonal anti-TNF antibody (CLB/7) (Sequin, Amsterdam, The Netherlands) and then incubated with 0.01 mg/ml recombinant human TNF-a (Partech, Rocky Hill, USA). Serial dilutions of serum samples and standard curve (0.1_50 nag/ml Ifx) were made in high performance ELISA (HPE) buffer (Sequin). Bound Ifx was detected with biotinylated affinity purified rabbit immunoglobulin G (IgG) to Fab regions of Ifx, and the reaction was developed with streptavidin-polyperoxidase (polyHRP) (Sequin). The detection limit of the assay was 1 nag/ml infliximab. Cut-off values were established with sera from 150 healthy blood donors and 100 RA patients who had never received Ifx (of whom 70% were RF positive) to exclude any background signal that might have been caused by RF or other auto-antibodies present in RA patient sera. Serum Ifx levels >10 nag/ml (mean + 6 S.D. control group) were considered positive.

Anti-infliximab Ab assay

ATI were detected by a two-site (bridging) ELISA, which takes advantage of the monovalency of the two arms of IgG subclasses 1, 2 and 3, to crosslink the Ifx coated on plates to biotinylated Ifx [11, 19]. Polystyrene plates (Nunc A/S, Roskilde, Denmark) were coated with Ifx (0.5 mg/ml) overnight. The following day, serial dilutions of samples (starting at 1/10) and a standard curve (0.48_250 AU/ml) diluted in HPE were incubated for 1 h with shaking. A standard curve was constructed using a patient serum that showed a high titre ATI (mainly IgG1) previously titrated in arbitrary units per millilitre by one of the authors 1446 of this study (L.A., data not shown). After washing, 10 nag/ml Ifx biotinylated by standard procedures (Pierce, Rockford, IL, USA), was added. Bound labeled Ifx was detected by incubation with polyHRP (1: 10 000) in PBS. The reaction was developed with tetramethylbenzidine (TMB)/H2O2 in 0.11M acetic acid buffer pH = 5.5 and stopped with 2M H2SO4. Washing steps were made in 0.01M PBS 0.02% Tween 20. The assay detection limit was 2 AU/ml and the cut-off for the presence of ATI in patient sera was established at 50AU/ml (mean + 6 S.D.) with the same control group used for the measurement of free Ifx. A linear dose-

response curve for inhibition was obtained when positive samples for ATI were pre-incubated with Ifx.

Statistical analysis

Descriptive statistics were provided using the mean, S.D., median (Mdn) and interquartile range (IQR). Statistical analysis was performed using the Statistical Package for the Social Sciences version 10.0 (SPSS, Chicago, IL, USA). Frequency data were compared by the Pearson's chi-square and Fisher's exact tests. Differences in quantitative values between groups were analyzed using Mann Whitney U and Wilcoxon non-parametric tests. Time course data were analyzed using the Kaplan Meier method. Statistical significance was calculated using the log-rank test and $P<0.05$ was considered statistically significant.

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Original article

Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis

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Abstract

Objective. To analyse the clinical relevance of the production of anti-infliximab antibodies (anti-infliximab Abs) in patients with RA undergoing infliximab treatment over a prolonged period of time.

Methods. Clinical characteristics, serum trough infliximab and antibody levels were evaluated in 85 RA patients treated with infliximab for a median of 4.42 (interval 0.4–10.2) years. DAS in 28 joints (DAS-28), EULAR response criteria and survival of treatment were assessed at 3 time points (6 months, 12 months and >4 years).

Results. Antibodies against infliximab were detected in 28 (32.9%) patients and were present in all EULAR non-responder patients. Antibody levels were higher in EULAR non-responders throughout the study period ($P = 0.05$ at 6 months, $P = 0.02$ at 1 year, $P = 0.003$ at >4 years) compared with EULAR (good and moderate) responders. Nine (10.5%) patients, all of them with high-serum anti-infliximab Ab levels, developed infusion-related reactions. Patients with anti-infliximab Abs more often required increased infliximab doses (51.7%) ($P = 0.032$) and median survival time on treatment was shorter (4.15 *vs* 8.89 years) ($P = 0.0006$). MTX co-therapy was not associated with lower proportion of anti-infliximab Ab-positive patients, but those receiving both infliximab and MTX had lower levels of anti-infliximab Abs ($P = 0.073$) and longer survival ($P = 0.015$) on treatment.

Conclusion. The formation of anti-infliximab Abs during treatment with infliximab is associated with a loss of clinical response, the appearance of infusion reactions and discontinuation of treatment.

Key words: Rheumatoid arthritis, Infliximab therapy, Immunogenicity, Efficacy, Long-term treatment.

Introduction

Since the approval of the first therapeutic mAb against TNF 15 years ago, the use of biological drugs in clinical practice has grown constantly [1]. The treatment of RA, Crohn's disease, psoriasis and other inflammatory diseases, which are usually refractory to conventional treatments, has improved considerably since combination regimens of these new biological drugs and the classical DMARDS were introduced [2].

Infliximab is a chimeric (mouse–human) mAb antagonist to TNF, and was the first antibody-based therapy to be introduced to treat patients with RA. Today its use has become more generalized and it is being administered to a growing number of patients at an early stage of disease, mainly because of its clinical efficacy and retarding effects on joint destruction [2]. Although the efficacy of this drug as a treatment for patients with active RA has been widely demonstrated [3, 4], some RA patients initially respond to treatment but subsequently their responsiveness decreases [1]. One of the alleged reasons for this phenomenon is immunogenicity associated with the drug itself. Infliximab can induce the formation of neutralizing

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antibodies [5], resulting in loss of efficacy and appearance of side effects such as infusion-related reactions [6, 7]. The induction of antibodies against the drug has been described in about half of the patients receiving repeated infliximab monotherapy; as a consequence, immune suppression by concomitant administration of MTX is recommended [4, 8, 9].

An antibody response to the drug often appears between the third and sixth month [10]. As long as the relative amount of the anti-drug antibodies is lower than the serum trough level of infliximab, the drug can provide a clinical benefit [11]. However, when the endogenous production of antibodies exceeds the amount of drug in the serum, the latter is cleared from the circulation [11, 12], the therapy is rendered ineffective and free antibodies to the drug can be measured in the patient's serum. The accelerated clearance of infliximab complexed to antibodies may result in decreased pharmacological availability [13, 14] and ultimately the loss of therapeutic effectiveness of infliximab [15]. Therefore, it is very likely that the equilibrium between infliximab and antibody response will regulate the overall effectiveness of the drug [11].

Most publications on the relationship between the presence of anti-infliximab antibodies (anti-infliximab Abs) and clinical response in RA patients focus on the first period of administration, with a maximum of 3 years follow-up [13]. Mulleman *et al*. [14] studied patients for >6 years, but only monitored the infliximab concentration. However, Wolbink and co-workers [11, 15] reported that development of the immune response against infliximab is a gradual process that may change over time because continuation of treatment may either induce immune tolerance or stimulate further antibody formation.

In this study, we present data on 85 RA patients undergoing infliximab treatment at the Rheumatology Unit of La Paz University Hospital since the end of 1999 (>10 years). Infliximab and anti-infliximab Ab levels were measured in order to assess the clinical relevance of infliximab immunogenicity throughout the course of the therapy.

Methods

Patients and sera

A total of 85 consecutive patients with RA, without previous biological treatment were included. Patients were enrolled at the Department of Rheumatology of La Paz University Hospital to receive infliximab therapy. This was a retrospective observational study, approved by the Hospital La Paz Ethics Committee and patients signed an informed consent form according to the Declaration of Helsinki. Serum samples (a total of 1451) were collected at the time of infusion, stored frozen and only thawed for the purpose of this study. The retrospective study period covers the years 1999 until 2010. All patients fulfilled the ACR 1987 revised criteria for RA and all of them had evidence of active disease, as indicated by a 28-joint DAS (DAS-28) at inclusion of 5.49 (1.2) [mean(S.D.)]. At first all patients were given i.v. infusions of 3 mg/kg infliximab at 0, 2, 6 and every 8 weeks thereafter. After 14 weeks of treatment, the rheumatologist was allowed to increase the infliximab dosage to 5 mg/kg depending on the observed clinical response. Every 6 months, disease activity using the DAS-28 and European League Against Rheumatism (EULAR) response criteria [16] was measured to assess clinical response. Six months, 1 year and >4 years [mean (S.D.) 5.9 (2) years] were chosen from the study as representative time points for patients' clinical response. Infusion reactions were defined as any event appearing during infusion requiring either arrest of drug infusion or the administration of parenteral medication.

Blood samples were collected at baseline and just before each infusion at 2, 6 and every 8 weeks thereafter, so that a maximum of 6–8 samples per year were obtained from each patient. Precise timing is required to compare results, because with a longer time interval serum infliximab may become undetectable due to normal drug pharmacokinetics, and not as a consequence of IC formation with anti-infliximab Ab. Sera were stored at −80°C until infliximab and anti-infliximab Abs were measured. At baseline, infliximab and anti-infliximab Ab concentrations in all patients were <10 ng/ml and 50 AU/ml, respectively.

Serum infliximab assay

Serum infliximab levels were determined by a sandwich ELISA, as described by Wolbink *et al*. [17] using a polyclonal anti-infliximab Ab [18]. Briefly, microtitre plates were coated with 2 μg/ml mouse monoclonal anti-TNF antibody (CLB/7) (Sanquin, Amsterdam, The Netherlands) and then incubated with 0.01 μg/ml recombinant human TNF-α (Peprotech, Rocky Hill, USA). Serial dilutions of serum samples and standard curve (0.1–50 ng/ml infliximab) were made in high performance ELISA (HPE) buffer (Sanquin). Bound infliximab was detected with biotinylated affinity purified rabbit immunoglobulin G (IgG) to Fab regions of infliximab, and the reaction was developed with streptavidin–polyperoxidase (polyHRP) (Sanquin). The detection limit of the assay was 1 ng/ml infliximab. Cut-off values were established with sera from 150 healthy blood donors and 100 RA patients who had never received infliximab (of whom 70% were RF positive) to exclude any background signal that might have been caused by RF or other auto-antibodies present in RA patient sera. Serum infliximab levels >10 ng/ml (mean + 6 S.D. control group) were considered positive.

Anti-infliximab Ab assay

Anti-infliximab Abs were detected by a two-site (bridging) ELISA, which takes advantage of the monovalency of the two arms of IgG subclasses 1, 2 and 3, to crosslink the infliximab coated on plates to biotinylated infliximab [11, 19]. Polystyrene plates (Nunc A/S, Roskilde, Denmark) were coated with infliximab (0.5 μg/ml) overnight. The following day, serial dilutions of samples (starting at 1/10) and a standard curve (0.48–250 AU/ml) diluted in HPE were incubated for 1 h with shaking. A standard curve was constructed using a patient serum that showed a high titre of anti-infliximab Ab (mainly IgG1) previously titrated in arbitrary units per millilitre by one of the authors

of this study (L.A., data not shown). After washing, 10 ng/ml infliximab biotinylated by standard procedures (Pierce, Rockford, IL, USA), was added. Bound labelled infliximab was detected by incubation with polyHRP (1 : 10 000) in PBS. The reaction was developed with tetramethylbenzidine (TMB)/H_2O_2 in 0.11 M acetic acid buffer pH = 5.5 and stopped with 2 M H_2SO_4. Washing steps were made in 0.01 M PBS 0.02% Tween 20. The assay detection limit was 2 AU/ml and the cut-off for the presence of anti-infliximab Ab in patient sera was established at 50 AU/ml (mean + 6 S.D.) with the same control group used for the measurement of free infliximab. A linear dose-response curve for inhibition was obtained when positive samples for anti-infliximab Abs were pre-incubated with infliximab.

Other autoantibodies

Antibodies to CCP (aCCP) were measured by ELISA (Eurodiagnostica, Malmö, Sweden), and RF was measured by nephelometry (Siemens, Marburg, Germany) with cut-off values of 25 and 9 UI/ml, respectively.

Statistical analysis

Descriptive statistics were provided using the mean, S.D., median (Mdn) and interquartile range (IQR). Statistical analysis was performed using the Statistical Package for the Social Sciences version 10.0 (SPSS, Chicago, IL, USA). Frequency data were compared by the Pearson's chi-square and Fisher's exact tests. Differences in quantitative values between groups were analysed using Mann–Whitney U and Wilcoxon non-parametric tests. Time course data were analysed using the Kaplan–Meier method. Statistical significance was calculated using the log-rank test and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 85 RA patients were enrolled in the study, of whom 69 were women, with a mean (S.D.) age of 53.8 (14.2) years at the beginning of infliximab treatment. Demographic and clinical characteristics are shown in Table 1. All patients received 3 mg/kg infliximab at baseline; however, 44 (51.8%) patients needed a gradual infliximab dose escalation by either increasing the dose to 5 mg/kg and/or shortening the interval between infusions, due to an inadequate response.

Clinical response and association with levels of infliximab and anti-infliximab Ab

At baseline, all patients had active disease as indicated by a mean (S.D.) DAS-28 of 5.49 (1.26) with no differences in DAS-28 values between patients that subsequently did [5.75 (1.28)], or did not [5.37 (1.25)] develop anti-infliximab Ab ($P = 0.204$). Anti-infliximab Abs were detected in serum samples from 28 (32.9%) patients, in all cases with undetectable serum trough infliximab levels. These antibodies appeared most frequently after the fourth infusion [Mdn 16 (range 14–79) weeks]; although in four patients

Table 1 Demographic and clinical characteristics of 85 RA patients

Variable	value
At study inclusion	
Age at onset, mean (S.D.), years	53.8 (14.2)
Gender: female, *n* (%)	69 (81)
aCCP positive, *n* (%)	69 (81)
RF positive, *n* (%)	67 (78)
During the study	
Concomitant anti-rheumatic therapy	
MTX alone, *n* (%)	29 (34)
MTX + other DMARDs, *n* (%)	40 (47)
Other DMARDs,[a] *n* (%)	15 (18)
None, *n* (%)	1 (1)
Concomitant use of glucocorticoids, *n* (%)	63 (74)
Time under infliximab, mean (interval), years	4.42 (0.4–10.2)
Infliximab discontinued, *n*/total (%)	45/84[b] (53.5)
Patients with acquired drug resistance, *n* (%)	44 (51.8)

[a]Other DMARDs: LEF, SSZ, HCQ and AZA. [b]The evolution of one patient was missed because she moved to another country.

the appearance of anti-infliximab Ab was delayed for >1 year. In most patients, antibody titres did not disappear, but increased during treatment and were only modulated by an increase in the dose of infliximab. Patients with antibodies against infliximab had higher DAS-28 values at 6-month follow-up (16 out of 49 patients), 1 year (7 out of 31 patients) and >4 years (11 out of 47 patients) [4.85 (1.24) *vs* 3.67 (1.12), $P = 0.004$; 4.95 (1.24) *vs* 3.13 (1.17), $P = 0.002$; 4.00 (1.35) *vs* 3.46 (1.22), $P = 0.004$, respectively]. Similar results were found for ΔDAS-28 from baseline [1.10 (0.93) *vs* 1.73 (1.03), $P = 0.044$; 1.24 (0.86) *vs* 1.92 (0.72), $P = 0.061$; 0.57 (1.86) *vs* 1.98 (1.26), $P = 0.025$] at the three time points, respectively.

Patients classified as responders were mainly patients with no detectable anti-infliximab Ab levels (Fig. 1). Only 24% of EULAR (good and moderate) responders ($n = 75$) showed anti-infliximab Ab *vs* 100% of non-responder patients ($n = 10$) ($P < 0.001$). Serum trough infliximab levels (Mdn, IQR) were higher in EULAR responders (good and moderate) than in EULAR non-responder patients at 6 months (992, 46–2960 *vs* 0, 0–60 ng/ml, $P = 0.005$), 1 year (1792, 384–3904 *vs* 0, 0–555 ng/ml, $P = 0.021$) and >4 years (1536, 220–3456 *vs* 0, 0–2672 ng/ml, $P = 0.101$), respectively (Fig. 2A). Serum anti-infliximab Ab concentration (Mdn, IQR) was higher in non-responders than in responders at 6 months (208, 0–1087 AU/ml *vs* 0, 0–100 AU/ml, $P = 0.054$), 1 year (60, 12–8924 *vs* 0, 0–0 AU/ml, $P = 0.018$) and >4 years (791, 0–6303 *vs* 0, 0–0, $P = 0.003$), respectively (Fig. 2B).

Survival of infliximab treatment

A total of 45 (53.5%) out of 84 patients interrupted infliximab therapy, with a median survival rate of 5.75 (95%

FIG. 1 Relationship between the presence of anti-infliximab Ab and EULAR response in RA patients treated with infliximab. Good: DAS-28 decrease >1.2 with an attained DAS-28 <3.2 Moderate: DAS-28 decrease ⩽1.2 and ⩾0.6 with an attained DAS-28 ⩾3.2 and ⩽5.1. No response: DAS-28 decrease <0.6 with an attained DAS-28 >5.1.

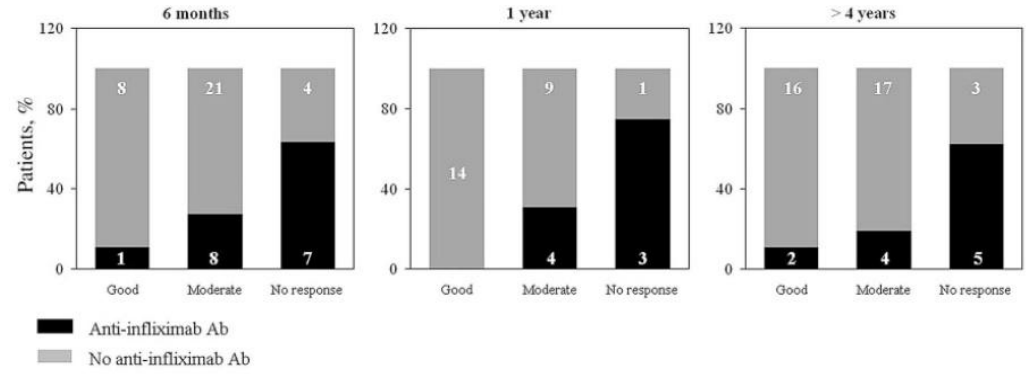
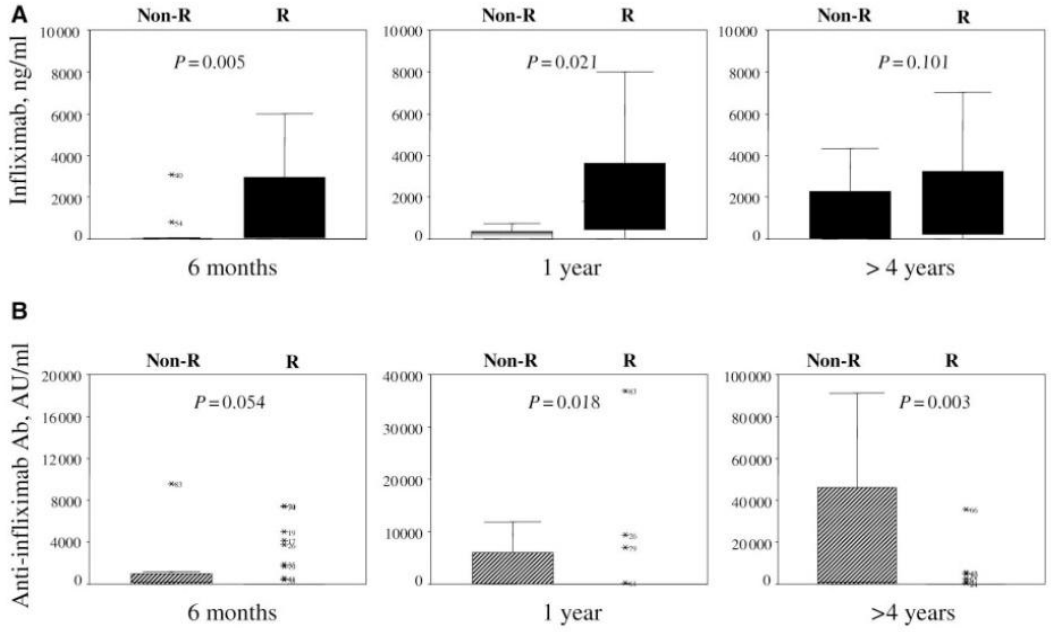


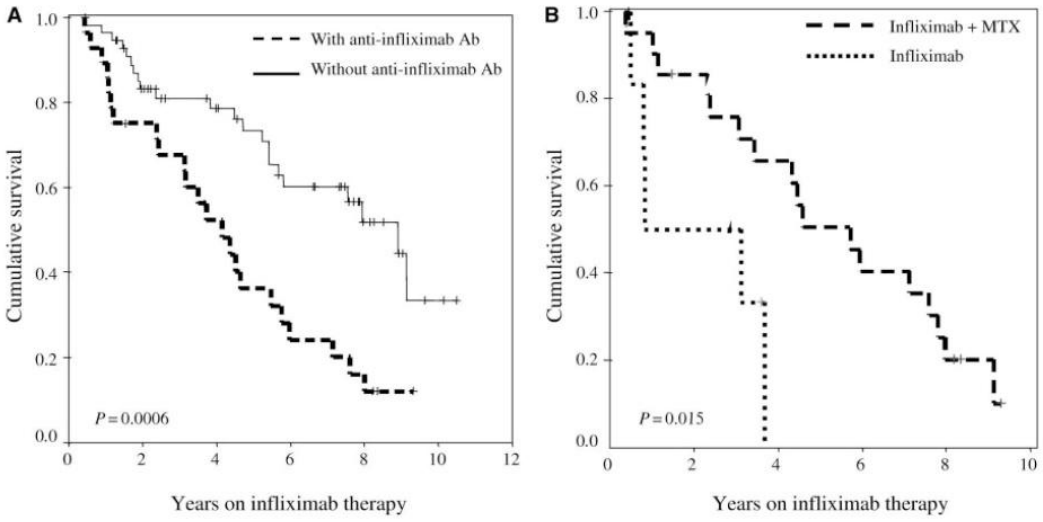
FIG. 2 Serum trough infliximab (**A**) and anti-infliximab Ab levels (**B**) in RA patients responding (R) (good and moderate) ($n = 38$ at 6 months, $n = 27$ at 1 year, $n = 39$ at >4years) and not responding (non-R) ($n = 11$ at 6 months, $n = 4$ at 1 year, $n = 8$ at >4 years) by EULAR criteria, to infliximab treatment. Data are shown as box plots, where the boxes represent the 25th to 75th percentiles, and the lines outside the boxes represent the 10th and 90th percentiles.



CI 4–7.5) years on the drug. The number of patients that discontinued infliximab therapy ($n = 45$) was significantly higher among those who developed anti-infliximab Ab [23 (82.1%) out of 28 *vs* 22 (39.3%) out of 57; $P < 0.001$]. Median survival time on infliximab treatment was 4.15 (95% CI 2.78–5.53) years in patients with anti-infliximab Ab *vs* 8.89 (95% CI 6.7–11) years for those without antibodies ($P = 0.0006$; Fig. 3A). Among patients who developed anti-infliximab Ab, median survival time on infliximab treatment was longer ($P = 0.022$) in patients receiving concomitant MTX ($n = 22$; 4.52 years, 95% CI 3.8–5.23 years) than in those not receiving MTX ($n = 6$; 1.06 years, 95% CI 0–3.79 years) (Fig. 3B).

Twenty-three (82.1%) of 28 patients who developed anti-infliximab Ab discontinued infliximab treatment. In four out of the five remaining patients, anti-infliximab

FIG. 3 Kaplan–Meier curves for survival on infliximab therapy of RA patients. (**A**) Patients who either developed (– –) or did not develop (——) anti-infliximab Ab. (**B**) Patients who developed anti-infliximab Ab and are treated with (– –) or without MTX (. . .).



Ab concentration decreased below detection levels after dosage escalation to 5 mg/kg, with a consequent clinical improvement, and the fifth patient continued treatment because her clinical response was good, despite remaining antibodies in circulation.

Modulation of anti-infliximab Ab levels by drug dose escalation

In 44 (51.7%) of the 85 patients, an acquired resistance to the drug was observed necessitating either an increased dosage of infliximab or a reduced time interval between infusions to achieve a clinical improvement. This drug resistance was higher in patients with anti-infliximab Ab [19 (67.9%) out of 28] than in those without anti-infliximab Ab [25 (43.9%) out of 57] ($P = 0.032$). Two kinds of response on dose escalation were observed in our cohort. Type I: anti-infliximab Ab disappeared after dose increase to 5 mg/kg coinciding with measurable infliximab serum trough levels and a DAS-28 decrease. In these cases, anti-infliximab Ab could be detected again if the dose of infliximab was subsequently reduced, with a simultaneous clinical worsening (Fig. 4A). Type II: anti-infliximab Ab did not disappear after drug escalation (Fig. 4B), reaching high levels, which in some patients were associated with development of infusion-related reactions (three patients).

Relation between infusion-related reactions and anti-infliximab Ab

Infusion-related reactions were recorded in nine patients, all of whom had detectable anti-infliximab Ab. Anti-infliximab Ab levels [Mdn (IQR)] at the time of infusion reaction were higher in the patients who developed reactions [20 565 (5000–30 625) AU/ml] than in those patients with detectable anti-drug antibodies, but without infusion-related reactions [10 152 (491–8162) AU/ml] ($P = 0.041$; Fig. 5).

Influence of combined therapy with MTX on anti-infliximab Ab presence

Sixty-nine (81.1%) patients received MTX [7.5–25 mg/weekly, mean (S.D.) 15 (4.96) mg/weekly] concomitantly with infliximab. MTX was subcutaneously and orally administered in 19 and 50 patients, respectively. We did not find a lower proportion of patients developing anti-infliximab Ab in association with the use of MTX (32% with MTX *vs* 37% without MTX, $P = 0.77$). However, in patients receiving MTX who did make antibodies ($n = 22$), maximal levels [Mdn (IQR)] tended to be lower than in those with antibodies on infliximab monotherapy ($n = 6$) [3414 (808–7426) AU/ml with MTX *vs* 21 250 (7049–47 656) AU/ml without MTX; $P = 0.07$].

Discussion

It is widely accepted that immunogenicity of biological drugs such as infliximab is the main cause of loss of clinical response in the treatment of RA [10, 13, 18, 20]. In this study, we have analysed the clinical significance of free infliximab and anti-infliximab Ab concentration in serum in a cohort of 85 Spanish RA patients treated for >4 years. Our findings indicate that one-third of RA patients develop antibodies and this is correlated with clinical response.

The variable incidence of anti-drug antibodies reported in earlier literature was mainly methodological and related to the method used to measure the antibodies [19]. Radioimmunoassay seems to be the most reliable and sensitive method to detect all antibody isotypes [5, 10, 18, 19, 21], but has the drawback of the use of radioactivity. The

FIG. 4 Modulation of serum infliximab and anti-infliximab Ab levels, as well as clinical response, with infliximab dose changes. Infliximab dose ranges between 3 and 5 mg/kg. (**A**) A 'type I' representative patient in whom anti-infliximab Ab levels are inhibited only by a high infliximab concentration. Lowering infliximab dose results in the appearance of antibodies and DAS-28 increase. (**B**) A 'type II' representative patient in whom anti-infliximab Abs do not disappear after infliximab dose increase, with a poor clinical response (DAS-28).

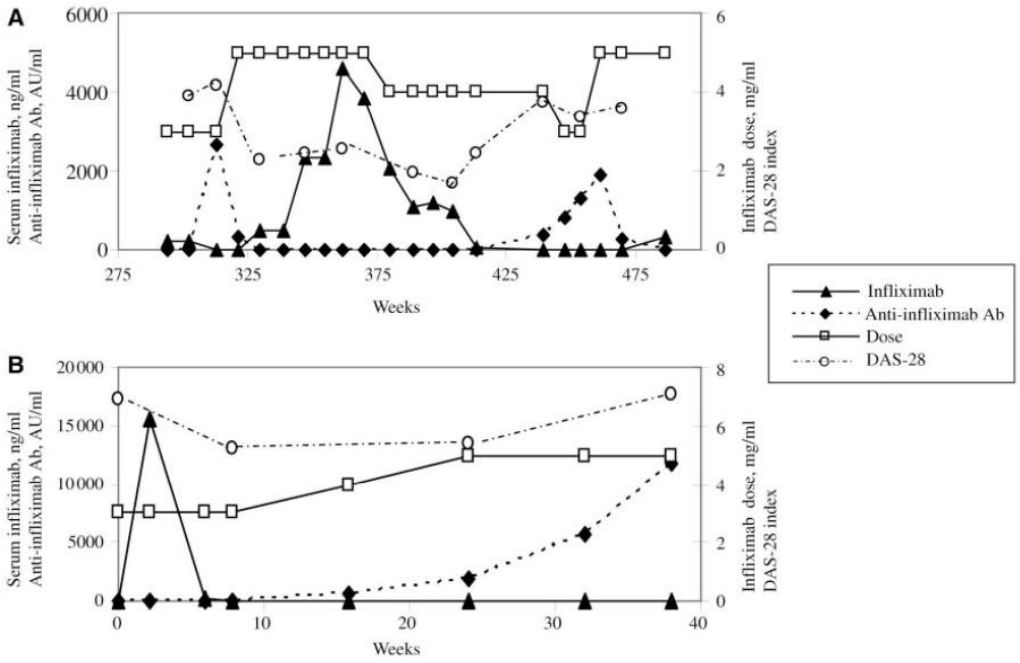
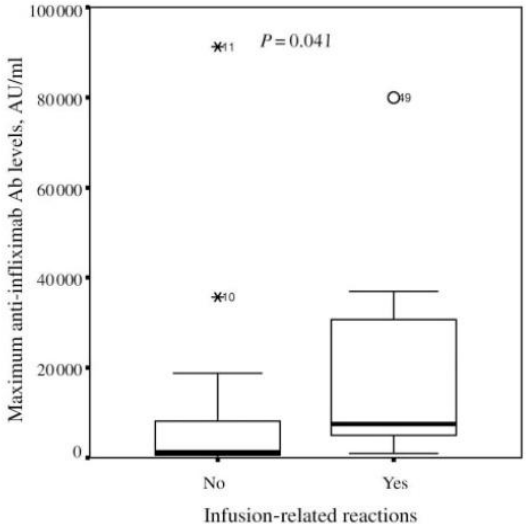


FIG. 5 Maximum antibody levels in patients with anti-infliximab Ab who developed or did not develop an infusion-related reaction, defined as in 'Material and methods' section.



bridging ELISA employed in this work suffers the disadvantage that it fails to measure IgG4 antibodies. However, this is not believed to represent a major problem as isolated IgG4 antibodies usually do not occur in the absence of other IgG isotypes [11] and the bridging ELISA approach for measuring anti-drug antibodies has been validated by its use in several other studies [11, 13, 20].

Serum levels of anti-infliximab Ab strongly correlate with the clinical response as DAS-28 was significantly lower in those patients without anti-infliximab Ab at all time points. According to the EULAR response criteria, 100% of non-responder patients at any studied time point showed anti-infliximab Ab *vs* only 24% of responders. Whereas most previously published studies were performed over relatively short periods of time ($\leqslant$1 year) [5–7], we have extended the analysis over >4 years, since we believe that immunogenicity rates can be underestimated if studies are restricted to <1 year. Moreover, during our long-term follow up, we have seen that patients with detectable levels of anti-infliximab Ab had to discontinue treatment earlier than those who did not develop anti-infliximab Ab.

As reported in previous literature, we have found that serum trough infliximab levels inversely correlate with the presence of antibodies against the drug and with the clinical response [10, 14, 17]. One could, therefore, argue

that there is no need to monitor both the drug and anti-drug antibodies, as complexes are formed between antibodies and infliximab [11]. However, undetectable or low levels of infliximab before the appearance of antibodies may indicate that the patient will develop a high titre of antibodies following the subsequent infusion. In fact, in our cohort one patient developed an infusion-related reaction during the fourth infusion, after a sharp decrease in circulating infliximab, but before antibodies could be detected.

The development of antibodies to infliximab occurred mainly in the first 4 months of treatment, although it can be delayed in patients with an early drug escalation, because only when the immune system makes sufficient antibodies to overcome the infliximab concentration is antibody detection possible. Detection of infliximab and anti-infliximab Ab levels can be used to customize treatment and help to avoid unnecessary therapy. As we have shown, patients differ in their clinical response to an increased dose of infliximab. In some patients, an improvement in DAS-28 was seen to coincide with measurable serum trough infliximab levels and loss of anti-infliximab Ab, which can reappear after dose decrease because of clinical improvement. In another group of patients, the antibodies remained in the circulation despite a drug dosage increase to maximum levels. These patients did not show clinical improvement and had a higher risk of developing infusion-related reactions. Another important utility of biopharmaceuticals monitoring by means of drug and anti-drug Ab determinations has been clearly exposed in a recent publication by Jamnitski *et al.* [22]. The authors show that among patients who discontinued treatment with a first TNF-α inhibitor, those who had developed antibodies against the drug achieved a significantly better clinical response after switching to another anti-TNF (etanercept) than patients without antibodies. Authors argue that immunogenicity monitoring is needed in order to differentiate patients who will benefit from a change in anti-TNF therapy from those who show no primary response.

Patients receiving infliximab treatment show a high rate of infusion-related reactions [5, 20]. In our cohort, all patients with infusion-related reactions had anti-infliximab Ab at high titres. These data support the view that elevated titres of anti-infliximab Abs are associated with increased risk of infusion reactions, probably because of the formation of large antibody complexes. These complexes are removed with difficulty by the liver and spleen, and are associated with the occurrence of serious adverse reactions [6].

It has been reported that combined therapy with infliximab and MTX is inversely associated with the formation of anti-infliximab Ab [23]. In our study, 81% of patients received concomitant MTX with infliximab and 32% of them developed anti-infliximab Ab. A similar number of patients developed antibodies when they were treated with infliximab alone or together with other DMARDs. These results, although similar to the findings reported by Haraoui *et al.* [13], were different from those observed in previous studies, which suggested that combined therapy reduced the number of patients developing anti-drug antibodies [4], probably due to differences in administration guidelines. However, in our study we observed that patients who developed anti-infliximab Ab continued on anti-TNF treatment significantly longer if they were receiving concomitant therapy with MTX, probably because the immunosuppression is associated with the production of lower antibody levels and the effectiveness of MTX itself on disease activity. This fact encourages us to recommend the routine use of MTX concomitantly with infliximab administration.

In conclusion, the formation of anti-infliximab Ab is associated with a poor clinical response and with the appearance of infusion reactions. Long-term follow-up shows that levels of these antibodies may be modulated by increasing drug concentration, which suggests that they may be used to monitor the appropriate therapeutic regime. Moreover, they are associated with the discontinuation of treatment over time.

Rheumatology key messages

- Immunogenicity of infliximab is associated with loss of clinical response and appearance of infusion reactions.
- Detection of anti-infliximab Ab can be used to customize treatment and help to avoid unnecessary therapy.
- Patients with anti-infliximab Ab discontinue infliximab treatment earlier than those who did not develop antibodies.

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ARTICLE 2

TITLE:*"Influence of immunogenicity on the efficacy of long-term treatment of spondyloarthritis with infliximab"*

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PATIENTS AND METHODS:

Patients and serum samples

A total of 94 patients with Spa, who had not received previous biological treatment, from SpA-Paz cohort were included. This was an ambispective observational study that was approved by the La Paz Hospital ethics committee. The patients signed an informed consent form. Serum samples (a total of 2116) were collected at the time of infusion. The retrospective study period covered the years from 1999 to 2008, and the prospective study period extended from 2009 to 2010. All of the patients with AS fulfilled the revised New York criteria. The patients with PsA fulfilled the GRAPPA (Group of Research and Assessment of Psoriasis and Psoriatic Arthritis) group criteria. All the patients with IBD fulfilled the ESSG (European Spondylarthropathy Study Group) criteria. At the time of inclusion, all patients had evidence of active disease, as indicated by their mean Ankylosing Spondylitis Disease Activity Score (ASDAS) of 3.08 (1.31) (mean (SD). All the patients were given intravenous infusions of 5 mg/kg IFX at 0, 2 and 6 weeks and then every 8 weeks thereafter. Every 6 months, disease activity was measured using the ASDAS (in the retrospective group of patients, the clinical response data were extrapolated from the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)).23 Six months, 1 year and >4 years (mean 5.9 years, SD 2 years) were chosen as the time points that would be used to represent the clinical course. Blood samples were collected at baseline and immediately before the 2- and 6-week

infusions and every 8 weeks thereafter. Serum samples from all patients at each of the three studied time points (6 months, 1 year and >4 years) could not be obtained.

Serum IFX assay

The serum Ifx levels were determined by a sandwich ELISA, as has been described previously. The cut-off values were established from the serum samples of 150 healthy blood donors and 100 patients with RA who had never received Ifx (70% of whom was RF positive). Serum Ifx levels >10 nag/ml (the mean + 6 SD of the control group) were considered positive.

Antibodies to infliximab (ATI) assay

ATI were detected using a two-site (bridging) ELISA as has been previously described. The cut-off point for the presence of ATI in patient serum samples was established at 50 AU/ml (the mean + 6 SD of the same control group used for the Ifx measurements).

Statistical analysis

The descriptive statistics consisted of the mean, SD, median (Mdn) and IQR. The statistical analyses were performed using the Statistical Package for the Social Sciences, version 10.0 (SPSS, Chicago, Illinois, USA). The frequency data were compared using the Pearson $\chi 2$ and Fisher exact tests. The continuous data were compared between groups using the Mann–Whitney U and Wilcoxon non-parametric tests. The time-course data were analyzed using the Kaplan–Meier method. Statistical significance was calculated using the log-rank test and p values <0.05 were considered significant.

EXTENDED REPORT

Influence of immunogenicity on the efficacy of long-term treatment of spondyloarthritis with infliximab

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ABSTRACT

Background Infliximab (IFX) is a monoclonal antibody against tumour necrosis factor α that is effective for treating spondyloarthritis (SpA). However, after initial success of the drug some patients lose responsiveness or develop infusion reactions, which may be related to the development of antibodies against the drug.

Objective To investigate the clinical relevance of antibodies to infliximab (ATI) formation in patients with SpA undergoing IFX treatment over a prolonged period.

Methods 94 patients with SpA treated with IFX from 1999 to 2010 were studied. Their clinical characteristics, serum trough IFX levels and ATI status were evaluated for a mean of 6.99 (95% CI:6.28 to 7.7) years. Clinical activity and improvement were measured using the Ankylosing Spondylitis Disease Activity Score (ASDAS): inactive <1.3, moderate ≥1.3 and <2.1, high ≥2.1–≤3.5, and very high >3.5 at three time points (6 months, 12 months and >4 years).

Results ATI were detected in 24 (25.5%) patients. The patients with ATI had higher ASDAS scores than those without ATI (2.55±0.89 vs 1.79±1.04, p=0.038 at 6 months; 1.95±0.67 vs 1.67±0.71, p=0.042 at 1 year; 2.52±0.99 vs 1.53±0.81, p=0.024 at >4 years). Eleven patients (12%) developed infusion-related reactions, and of these, ATI were present in eight patients (73%). The patients with infusion-related reactions had higher ATI titres (median 12 931 AU/ml, IQR 853–82 437) vs median 2454 AU/ml, IQR 449–7718, p=0.028) and shorter survival (4.25 years vs 8.19 years, p<0.001). ATI development occurred more frequently in the patients not receiving methotrexate (20/58 (34.5%) vs 4/36 (11.1%), p=0.011).

Conclusion In patients with SpA treated with IFX, ATI formation is associated with a poor clinical response, the appearance of infusion reactions and the discontinuation of treatment.

INTRODUCTION

Spondyloarthritis (SpA) is a heterogeneous group of diseases consisting of ankylosing spondylitis (AS), psoriatic arthritis (PsA), arthritis related to inflammatory bowel disease (IBD), reactive arthritis, a subgroup of juvenile idiopathic arthritis and undifferentiated spondyloarthritis.[1] Until recently, the main treatment for SpA was based on non-steroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs (DMARDs) and physical therapy. Although DMARDs such as methotrexate (MTX) and sulfasalazine can be useful for treating the peripheral joint manifestations of SpA, they have no demonstrated efficacy for treating the axial manifestations[2–5]; however, several studies have demonstrated the efficacy of biological drugs such as antitumour necrosis factor α (anti-TNFα) for treating patients with SpA.[6–13]

All biological drugs can induce an immune response, with the structure of the anti-TNF agent being a decisive factor in its immunogenicity. Infliximab (IFX) is a chimeric monoclonal IgG1 antibody against TNF that has been approved for treating moderate to severe SpA. The drug elicits a response that is inferior to that obtained with conventional treatment. Although the efficacy of IFX against SpA has been shown in large, randomised clinical trials,[14–17] it is known that >30% of patients with AS fail to respond or lose their responsiveness.[18] Part of this treatment failure can be explained by the development of antidrug antibodies.[18–20]

Until now, few studies have been published on treating SpA with IFX. Two of these studies have been conducted in patients with AS and have investigated the clinical response in relation to antibodies to IFX (ATI) formation after ≥1 year.[18 20] In this study, we analysed the clinical consequences of ATI formation in a group of patients with SpA (some of whom had conditions other than AS) who were treated with IFX over long periods. At the same time, there is increasing interest in examining the influence of MTX on patients with SpA treated with IFX.[21 22] In our group of patients with SpA, we assessed whether concomitant immunosuppressive treatment with MTX plays a role in ATI production.

PATIENTS AND METHODS

Patients and serum samples

A total of 94 patients with SpA (50 AS, 12 undifferentiated spondyloarthritis, 22 PsA and 10 SpA associated with IBD) who had not received previous biological treatment were included. The patients were enrolled at the department of rheumatology of La Paz University Hospital. This was an ambispective observational study that was approved by the La Paz Hospital ethics committee. The patients signed an informed consent form. Serum samples (a total of 2116) were collected at the time of infusion. The retrospective study period covered the years from 1999 to 2008, and the prospective study period extended from 2009 to 2010. All of

the patients with AS fulfilled the revised New York criteria. The patients with PsA fulfilled the GRAPPA (Group of Research and Assessment of Psoriasis and Psoriatic Arthritis) group criteria. All the patients with IBD fulfilled the ESSG (European Spondylarthropathy Study Group) criteria. At the time of inclusion, all patients had evidence of active disease, as indicated by their mean Ankylosing Spondylitis Disease Activity Score (ASDAS) of 3.08±1.31 (mean ± SD). All the patients were given intravenous infusions of 5 mg/kg IFX at 0, 2 and 6 weeks and then every 8 weeks thereafter. Every 6 months, disease activity was measured using the ASDAS (in the retrospective group of patients, the clinical response data were extrapolated from the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)).[23] Six months, 1 year and >4 years (mean 5.9 years, SD 2 years) were chosen as the time points that would be used to represent the clinical course. Infusion reactions were defined as events appearing during infusion that required either cessation of the drug infusion or the administration of parenteral medication.

Blood samples were collected at baseline and immediately before the 2- and 6-week infusions and every 8 weeks thereafter. Precise timing was required to compare the results because serum IFX may become undetectable over longer time intervals owing to normal drug pharmacokinetics rather than the formation of ICs with ATI. Therefore, samples taken more than 9 weeks after the previous infusions were not used in the study. The serum samples were stored at −80°C until measurement of the IFX and ATI. Serum samples from all patients at each of the three studied time points (6 months, 1 year and >4 years) could not be obtained. We obtained samples from 56 patients immediately after they had begun treatment, from nine patients in the first year but after starting IFX treatment and from 29 patients after the first year of anti-TNF treatment. A minimum of five samples and a maximum of 32 samples per patient were collected. In all patients from whom baseline samples were available, the IFX and ATI concentrations were <10 ng/ml and 50 arbitrary units per millilitre (AU/ml), respectively.

Serum IFX assay

The serum IFX levels were determined by a sandwich ELISA, as has been described previously.[24 25] The cut-off values were established from the serum samples of 150 healthy blood donors and 100 patients with rheumatoid arthritis (RA) who had never received IFX (70% of whom were rheumatoid factor positive). Serum IFX levels >10 ng/ml (the mean + 6 SD of the control group) were considered positive.

Antibodies to infliximab (ATI) assay

ATI were detected using a two-site (bridging) ELISA as has been previously described.[25] The cut-off point for the presence of ATI in patient serum samples was established at 50 AU/ml (the mean + 6 SD of the same control group used for the IFX measurements).

Statistical analysis

The descriptive statistics consisted of the mean, SD, median (Mdn) and IQR. The statistical analyses were performed using the Statistical Package for the Social Sciences, version 10.0 (SPSS, Chicago, Illinois, USA). The frequency data were compared using the Pearson χ^2 and Fisher exact tests. The continuous data were compared between groups using the Mann–Whitney U and Wilcoxon non-parametric tests. The time-course data were analysed using the Kaplan–Meier method. Statistical significance was calculated using the log-rank test, and p values <0.05 were considered significant.

RESULTS

Patient characteristics

A total of 94 patients with SpA were enrolled in the study, of whom 53 (56.4%) were men, with a mean (±SD) age of 50±11 years. Their demographic and clinical characteristics are shown in table 1. Of the 94 patients with SpA, 56 were analysed at 6 months (44 without ATI and 12 with ATI), 51 at 1 year (41 without ATI and 10 with ATI) and 56 at >4 years (44 without ATI and 12 with ATI). All of the patients received the standard regimen of 5 mg/kg IFX every 8 weeks; however, 18 (19%) of them needed more frequent infusions because the response obtained was inadequate.

Clinical response and association with serum IFX and ATI levels

ATI were detected in the serum samples of 24 (25.5%) patients, all of whom had undetectable serum trough IFX levels. In most of these patients, the antibodies appeared after the sixth infusion (median 44, IQR 24–55 weeks). In 17/24 (71%), the ATI occurred in the first year of IFX treatment, and in three (12.5%) patients, the antibodies were detected after 2 years (see online supplementary figure S1). We could not determine the exact moment of ATI development in four of the 24 patients because their initial samples were positive.

All patients had active disease at baseline, as indicated by a mean ASDAS of 3.08±1.31, with no differences in ASDAS values between the patients who subsequently did (2.94±0.72) or did not (3.14±1.46) develop ATI (p=0.534). The patients with ATI had significantly higher clinical activity (as measured by the ASDAS) at 6 months (2.55±0.89 vs 1.79±1.04, p=0.038), 1 year (1.95±0.67 vs 1.67±0.71, p=0.042) and >4 years (2.52±0.99 vs 1.53±0.81, p=0.024) of follow-up (figure 1A). The change in ASDAS (ΔASDAS) values was lower in the group of patients who developed antibodies at any time (0.48±0.73 vs 1.47±1.66, p=0.029 at 6 months; 0.81±1.20 vs 1.56±1.67, p=0.098 at 1 year; 0.45±0.82 vs 1.43±1.25 p=0.022 at >4 years) (figure 1B). The patients with ATI had less pronounced clinical improvement by ASDAS criteria at 6 months (13% vs 87%, p=0.077), 1 year (9.1% vs 90.9%, p=0.105) and > 4 years (8.7% vs 91.3%, p=0.05). During the study, 51 patients (54.3%) showed clinically

Table 1 Demographic characteristics of patients

Characteristics	Value
Men, n (%)	53 (56.4)
Age (years), mean (SD)	50 (11)
HLA positive, n (%)	17/29 (58.6)
Clinical manifestation, n (%)	
Only axial	63 (67)
Axial and peripheral	31 (33)
Concomitant treatment, n (%)	
Methotrexate (MTX)	21 (22.3)
Others DMARDs (OD)	25 (26.6)
MTX+OD	26 (27.7)
Monotherapy	22 (23.4)
Corticosteroid treatment	38/91 (41.8)
Time in years receiving IFX treatment, mean (95% CI)	6.99 (6.28 to 7.7)
Patients with ATI, n (%)	24 (25.5)

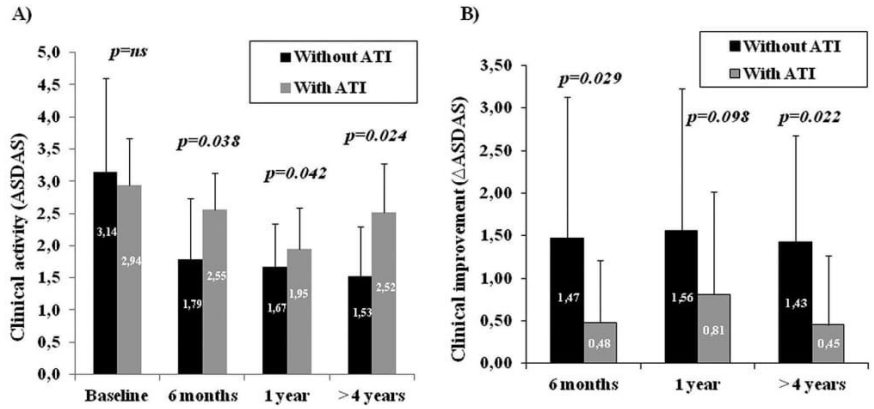

Figure 1 (A) Association between clinical activity, as measured by the ASDAS, and ATI development. ASDAS values (mean ± SD) at baseline, 6 months, 1 year and >4 years in the patients who developed ATI (□) and in those who did not develop ATI (■). (B) Association between clinical improvement, as measured by ASDAS (mean ± SD), and the absence (■) or presence (□) of ATI in the patients with SpA. ASDAS, Ankylosing Spondylitis Disease Activity Score; ATI, anti-infliximab antibody; SpA, spondyloarthritis.

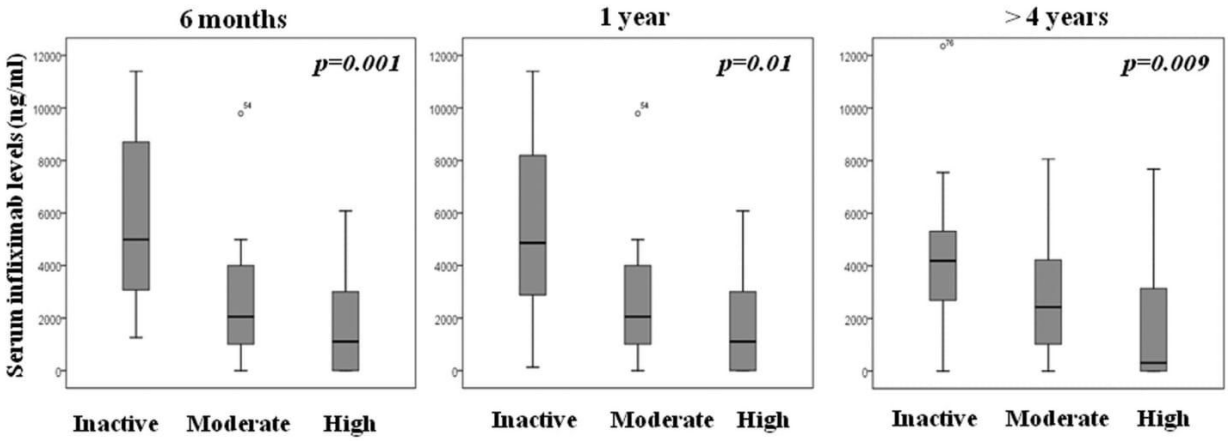

Figure 2 The association between clinical activity as measured by ASDAS (inactive, moderate and high/very high activity) and infliximab levels at 6 months, 1 year and >4 years. The data are presented as interquartile ranges (75th centile, upper edge; 25th centile, lower edge; and 50th centile, midline of the box). ASDAS, Ankylosing Spondylitis Disease Activity Score.

significant improvement, only nine (17.6%) of whom developed ATI (p=0.047).

The patients with inactive disease came mainly from the group with no detectable ATI (43.5% without ATI vs 0% with ATI, p=0.001 at 6 months; 31.4% without ATI vs 12.5% with ATI, p=0.091 at 1 year; and 48.7% without ATI vs 9% with ATI, p=0.001 at >4 years). Patients with inactive disease had higher IFX levels (median, IQR) than those with active disease (inactive: 4992, 2976–8768 ng/ml vs moderate: 2048, 840–4112 ng/ml vs high: 1104, 0–3150 ng/ml, p=0.001 at 6 months; inactive: 4128, 2768–7824 ng/ml vs moderate: 2336, 852–3568 ng/ml vs high: 1328, 0–3366 ng/ml, p=0.010 at 1 year; inactive: 4192, 2872–5344 ng/ml vs moderate: 2432, 636–4288 ng/ml vs high: 308, 0–3296 ng/ml, p=0.009 at >4 years) (figure 2). Consequently, the ATI levels (median, IQR) were lower in the patients in remission (0, 0–0 AU/ml vs 0, 0–672 AU/ml, p=0.006 at 6 months; 0, 0–0 AU/ml vs 0, 0–447 AU/ml, p=0.074 at 1 year; 0, 0–0 AU/ml vs 0, 0–577 AU/ml, p=0.018 at >4 years).

Modulating ATI levels by shortening the intervals between infusions

In 18 (19%) of the 94 patients, the time between infusions was shortened owing to insufficient responses. This change was required more frequently in the patients with ATI (8/24 (33.3%) vs 10/70 (14.3%); p=0.044, respectively). A clinically significant improvement was seen more often in the patients without ATI (8/10 (80%) vs 2/8 (25%), p=0.031, respectively). In four patients (50%), the ATI levels were reduced to negative levels after shortening the infusion intervals. Moreover, the ATI levels of these four patients before the more frequent infusions were lower than those of the patients in whom ATI remained detectable after shortening the interval between infusions (median, IQR) (121.5, 68.5-191.0 vs 30000, 1325-41625; p=0.014, respectively).

Survival analysis for the IFX treatment

Twenty-seven (28.7%) of the 94 patients interrupted their IFX treatment, mainly owing to an insufficient response or the

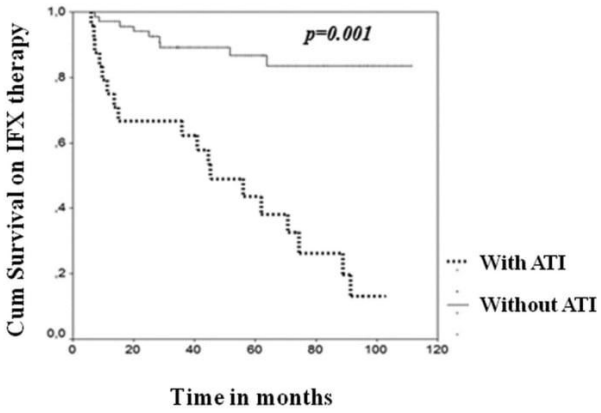

Figure 3 The infliximab survival curve in the patients with spondyloarthritis with/without ATI. Mean drug survival was shorter in the patients with ATI than in those without ATI (4.25 years, 95% CI 3.06 to 5.43 vs 8.19 years, 95% CI 7.54 to 8.85; $p=0.001$).

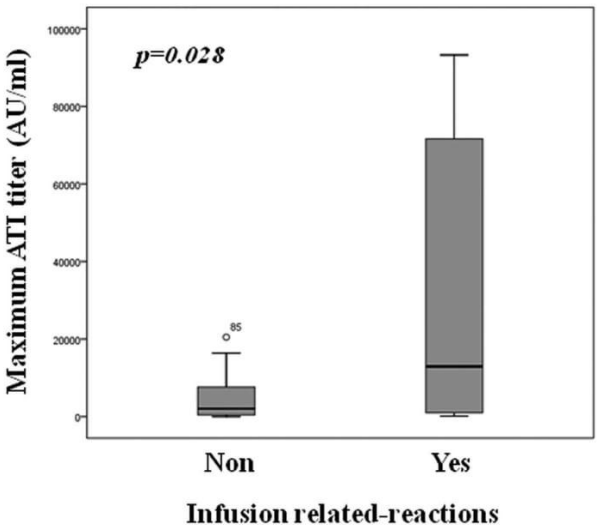

Figure 4 The maximum anti-infliximab antibody (ATI) levels in the patients who developed or did not develop infusion-related reactions. The data are presented as interquartile ranges (75th centile, upper edge of the box; 25th centile, lower edge of the box; 50th centile, midline of the box).

development of infusion-related reactions, with a median survival time of 6.99 years (95% CI 6.28 to 7.7). A larger fraction of the patients with ATI discontinued their IFX treatment (18/24 (75%) vs 9/70 (12.8%); p<0.001). The median IFX survival time was 4.25 years (95% CI 3.06 to 5.43) in the patients with ATI versus 8.19 years (95% CI 7.54 to 8.85) in the patients without ATI (p<0.001) (figure 3). The patients with ATI who did not discontinue the biological treatment had significantly lower antibody levels (0, 0–0 AU/ml vs 577, 0–5887 AU/ml; p=0.005) than did the patients who discontinued.

Relationship between ATI and infusion-related reactions

Infusion-related reactions were seen in 11 of 94 patients, most of whom had detectable ATI (8 (72.7%) vs 3 (27.3%), p=0.001). The ATI levels (median, IQR) at the times of the infusion reactions were significantly higher in the patients who developed infusion reactions (12 931, 853–82 437 AU/ml vs 2454, 449–7718 AU/ml; p=0.028) (figure 4).

Influence of combined treatment with MTX on ATI presence

A total of 47 out of 94 (50%) patients received concomitant MTX treatment (mean ± SD) (15±4.96 mg/weekly) sometime during the study, but only 36/94 (38%) patients were taking MTX before starting anti-TNF treatment. ATI were detected before starting MTX in nine of the remaining 11 patients; therefore, they were included in the group not taking MTX for the purposes of the statistical analysis. ATI developed more frequently in the patients not taking MTX (20/58 (34.5%) of those not taking MTX vs 4/36 (11.1%) of those taking MTX, p=0.011). In the four patients taking MTX who developed ATI, the appearance of antidrug antibodies occurred later (70.83±62.2 weeks in patients receiving IFX+MTX vs 36.50±16.58 weeks in patients receiving IFX alone, p=0.148). Moreover, the maximum IFX levels (mean ± SD) tended to be higher in the patients with concomitant MTX treatment (4548.26±3832.30 vs 3484.97±3018.47, respectively, p=0.147).

DISCUSSION

This study shows that ATI formation in patients with SpA occurs most often after 6 months of anti-TNF treatment, although it can occur as long as 2 years after beginning IFX. The appearance of ATI is associated with a diminished clinical response, the development of infusion-related reactions, and a higher likelihood of needing more frequent infusions and discontinuing treatment. Concomitant MTX treatment also protects against the formation of ATI.

All biological drugs can induce an immune response, with the structure of anti-TNF agents being a decisive factor in their immunogenicity. The percentage of patients who develop ATI varies among different chronic inflammatory diseases. ATI have been seen in 12–44% of patients with RA,[26–32] in 6–55% of patients with Crohn´s disease[33–37] and in up to 29% of patients with AS.[18] Studies have demonstrated that chimeric drugs, which have murine components, have a greater likelihood of inducing antidrug antibody development than do fully human antibodies.[38] The question that remains is why all patients treated with anti-TNF agents do not develop antidrug antibodies. Immunogenicity seems to be the result of several factors associated with the treatment, the patient and external factors,[35 39 40] such as dose, treatment formulation, assay technology, contaminants in the host cells, genetic background and concomitant treatment with immunosuppressive drugs.[35 40–43]

In our group's previous study of patients with RA treated with IFX,[24] we found that ATI development occurs most frequently within the first 4 months (median 16 weeks; IQR 14–79) of treatment; whereas in patients with SpA, it usually occurs after 6 months. Patients with SpA are treated with higher IFX doses (5 mg/kg) from the onset of treatment; therefore, greater antibody production would be needed to neutralise the higher IFX concentration,[24 44] and immunotolerance is more likely to develop.[41] This observation suggests that the frequency of ATI formation may have been underestimated in previous studies of immunogenicity in SpA with short observation times.[18 20–22] Another factor that may underestimate the immunogenicity of IFX is the type of assay used to detect ATI (radioimmunoassay or ELISA) and the timing of sample extraction.

It is well known that the appearance of antibodies against a drug has a negative effect on the clinical response to that drug.[18 24 45 46] A clinically significant improvement was not seen in 15 (62.5%) of the 24 patients with ATI. Moreover, the patients with ATI had significantly greater clinical activity at all the observation time points, and only a minority of the patients with ATI achieved remission. In previous publications on patients with SpA, the appearance of antidrug antibodies has also been associated with a poor clinical response.[18 20 45] Conversely, more patients with measurable serum trough IFX levels reported clinical improvement during the study, and higher IFX levels were associated with inactive disease at any time point studied. Consistent with our results, de Vries *et al* observed a positive correlation between clinical response and IFX levels in 38 patients with AS treated with IFX for 54 weeks.[18] Similar findings have also been described with other biological agents, such as adalimumab[45] and etanercept.[47] As we have shown, IFX levels are closely correlated with clinical activity, and the presence of ATI should be suspected when low or undetectable IFX levels are found.

In 19% of the patients, the interval between infusions was shortened to 6–7 weeks because of an insufficient response. Most of the patients without ATI (80%) achieved a significant clinical improvement with the more frequent infusions. Four out of eight patients with ATI achieved undetectable ATI after more frequent infusions and two of these patients showed significant clinical improvement with the adjusted therapeutic regimen. This result is probably due to the higher free serum IFX levels attained. Higher serum levels would neutralise ATI after increasing the dose or shortening the interval between infusions as previously described.[44] Other authors have reported a lack of clinical improvement in patients with AS treated with IFX when the drug infusion interval was modified in patients with ATI[2]. We suggest that any modification in the therapeutic regimen (a dose increase or more frequent infusions) that is not followed by neutralisation of the antibody levels may not provide clinical benefits. Therefore, monitoring ATI levels should play an important role in avoiding the continuation of ineffective treatment.

The appearance of infusion-related reactions is one of the most important side effects of IFX treatment in different inflammatory diseases, as has been widely described.[22 24 29 48] In our cohort, most of the patients with infusion-related reactions had high ATI levels (72.7%), which is similar to the results of our previous study in RA patients treated with IFX.[24] Ducourau *et al* also found a strong correlation between ATI development and the appearance of an infusion-related reaction.[22]

Several studies have suggested that patients with RA treated with combined treatment (IFX and MTX) have less frequent ATI development[22 29] or that they develop lower ATI levels.[24 32] In our study, we found a significantly reduced rate of ATI development in the patients receiving combined treatment, as has been recently reported by others.[22] Moreover, we found that the appearance of ATI was delayed in the patients receiving MTX. These findings may explain why, in some recent studies,[21 49] no influence of MTX on IFX exposure has been found among patients with AS. The authors measured IFX concentrations for no longer than 18[21] or 22[50] weeks, at which point, in our experience, most patients with SpA will not have developed ATI. Contrary to the reports of some previous studies,[21 49] we found that the maximum IFX levels tended to be higher in the patients with concomitant MTX treatment. This finding suggests that combined MTX treatment has a significant anti-immunogenic effect in preventing ATI development,[51] which results in higher serum IFX levels.

In conclusion, ATI development is associated with a poor clinical response, the discontinuation of treatment and an increased incidence of adverse effects. Long-term follow-up demonstrates that ATI may form at any time, resulting in secondary inefficacy. Therapeutic regimen modulation seems to be more useful for achieving a clinical improvement in patients without ATI. Our results suggest that combined MTX treatment is useful for avoiding ATI development.

Contributors CP and DP-S wrote the article. LN, GB, DP, AV, CP and AB evaluated the patients. AB and EM-M reviewed the article. RM, DN and ARdA carried out laboratory procedures. JD carried out the statistical analysis.

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Competing interests AB has received fees from Roche, Schering-Plough, Wyeth, Abbott, BMS and USB. EM-M is a consultant and a member of speakers' bureaus for Pfizer, MSD, UCB and Abbott. CP, DP-S, GB and LN have received speaker honoraria from Pfizer. All other authors have declared no conflicts of interest.

Patient Consent Obtained.

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Influence of immunogenicity on the efficacy of longterm treatment of spondyloarthritis with infliximab

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ARTICLE 3

TITLE: ***"The timing of serum infliximab loss or the appearance of antibodies to infliximab (ATI) is related with the clinical activity in ATI-positive patients with rheumatoid arthritis treated with infliximab"***

JOURNAL: Ann Rheum Dis 2013;72(11):1888–90(103)

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PATIENTS AND METHODS:

Eleven ATI-positive patients with RA receiving Ifx every 8 weeks were included. Disease activity was assessed by DAS28 and European League Against Rheumatism (EULAR) criteria at baseline, and during weeks n and n+8. The serum drug and ATI levels were determined using a capture and bridging ELISA, as previously described, at weeks n, n+4 and n+8. Patients were divided according to the findings from week n+4: Group 1 (ATI-positive=without Ifx and ATI-positive, ATI-negative=with or without Ifx and ATI-negative); Group 2 (with Ifx=ATI-negative and with Ifx, without Ifx=ATI-positive or ATI-negative and without Ifx).

The timing of serum infliximab loss, or the appearance of antibodies to infliximab (ATI), is related with the clinical activity in ATI-positive patients with rheumatoid arthritis treated with infliximab

In patients with rheumatoid arthritis (RA), the development of antibodies to infliximab (ATI) is associated with poor clinical response.[1 2 7] Nevertheless, there is no plausible explanation for why not all patients with ATI experience high disease activity. To investigate whether the timing of ATI appearance and/or drug disappearance is correlated with clinical activity, we measured the infliximab (Ifx) and ATI levels in patients 4 weeks after infusion (n+4).

Eleven ATI-positive patients with RA receiving Ifx every 8 weeks were included. Disease activity was assessed by DAS28 and European League Against Rheumatism (EULAR) criteria at baseline, and during weeks n and n+8. The serum drug and ATI levels were determined using a capture and bridging ELISA, as previously described,[1 6 8 10] at weeks n, n+4 and n+8.

Patients were divided according to the findings from week n+4: Group 1 (ATI-positive=without Ifx and ATI-positive, ATI-negative=with or without Ifx and ATI-negative); Group 2 (with Ifx=ATI-negative and with Ifx, without Ifx=ATI-positive or ATI-negative and without Ifx). Table 1 shows the patients demographic and clinical characteristics. At week n+8, four patients were moderate responders and seven patients were non-responders. In week n+4, four patients had ATI but did not have Ifx, two patients had no ATI but did have Ifx, and five patients had neither ATI nor Ifx.

ATI status in week n+4 and clinical activity in week n+8 was as follows. Four patients were ATI-positive (36.3%) in week n+4, and were non-responders in week n+8. ATI-positive patients

Table 1 Demographic characteristics of 11 patients with rheumatoid arthritis (RA)

	Total: 11 patients with RA (%)
Gender, female n (%)	11 (100)
Age, mean (SD)	54.18 13.24
Autoantibodies:	
RF positive n (%)	9 (81.8)
ACPA positive n (%)	9 (81.8)
Disease duration (years), mean (SD)	14.20 6.42
Baseline DAS28, mean (SD)	5.45 1.13
Time under Ifx therapy in years, mean (SD)	8.00 2.75
ATI duration in years, mean (SD)	1.1 0.91
Concomitant treatment	
MTX alone	3 (27.2)
OD	1 (9.0)
MTX+OD	6 (54.8)
Ifx monotherapy	1 (9.0)

ACPA, Anticitrullinated protein antibody; ATI, antibodies to infliximab; DAS28, Disease Activity Score 28; Ifx, infliximab; MTX, Methotrexate; OD, Other DMARDs; RF, Rheumatoid Factor.

had higher DAS28 (6.56 0.69 ATI-positive vs 4.56 0.93 ATI-negative, p=0.012) and lower clinical improvement in week n+8 (−1.22 0.72 ATI-positive vs 0.94 0.93 ATI-negative, p=0.012) (figure 1). DAS28 and ATI levels were statistically correlated (r=0.662, p=0.026 using Pearson s correlation coefficient (PCC)). ATI levels in week n+8 were higher in ATI-positive patients in week n+4 (Mdn, IQR 8037.5, 2640.0 12 062.5 AU in ATI-positive vs 260.0, 131.0 342.0 AU in ATI-negative in week n +4, p=0.024). ATI levels in week n+4 were lower than ATI levels in week n+8 (Mdn, IQR 0.0, 0.0 1306.0 AU in week n+4 vs 342.0, 186.0 5000.0 AU in n+8, p=0.045).

Ifx serum levels in week n+4 and clinical activity in week n+8. Two patients (18.1%) had Ifx in week n+4 and were classified as moderate responders. These patients had lower DAS28 than patients without Ifx (3.54 0.02 with Ifx vs 5.68 1.08 without Ifx, p=0.036) (figure 1). The clinical improvement was lower in patients without Ifx in week n+4, although these data were not statistically significant (1.23 1.15 with Ifx vs −0.09 1.41 without Ifx, p=0.232) (figure 1). DAS28 and Ifx levels were statistically correlated (r=−0.661, p=0.027, by PCC).

We demonstrated that the disease activity and ATI levels before the Ifx infusion were correlated with the moment of drug disappearance or ATI appearance. At week n+4, ATI levels were observed in most clinically active patients. These findings reflect that clinical efficacy is related to the persistence of circulating Ifx levels. Logically, patients with higher ATI levels neutralised Ifx earlier, thereby preventing the effect of Ifx. Higher ATI levels detected just before the infusion may have resulted from an earlier ATI development after drug infusion or a faster drug clearance.

Five patients with RA had no ATI or Ifx levels in week n+4, most likely because Ifx is in complex with ATI, as suggested by Wolbink *et al*.[10]

In conclusion, this study shows that the effect of Ifx in ATI-positive patients is correlated with the timing of the drug disappearance and ATI appearance between infusions, each resulting in different clinical consequences. Another finding is that higher ATI levels are found in patients with earlier drug clearances. Further studies with larger patient groups are needed to confirm the clinical relevance of these findings.

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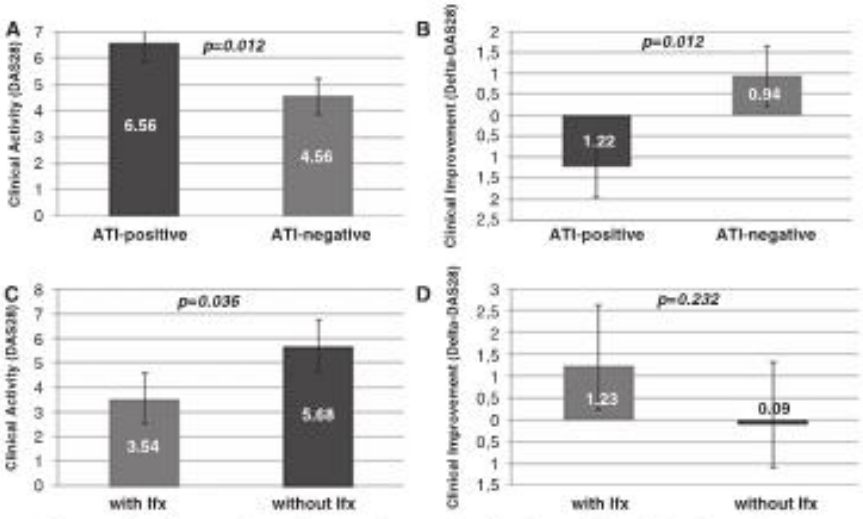

Figure 1 (A) Association between clinical activity (DAS28) in week n+8 and antibodies to infliximab (ATI) status (positive/negative) in week n+4. The clinical activity was measured by DAS28 (mean SD) between ATI positive or negative patients in the middle of the infusion cycle. (B) Association between clinical improvement (δ-DAS28) in week n+8 and ATI status in week n+4. The clinical improvement was measured by delta-DAS28 (mean SD) between ATI positive or negative patients in the middle of the infusion cycle. (C) Association between clinical activity (DAS28) in week n+8 and infliximab (Ifx) status (with/without) in week n+4. The clinical activity was measured by DAS28 (mean SD) between the patients with or without detectable Ifx levels in the middle of the infusion cycle. (D) Association between clinical improvement (delta-DAS28) in week n+8 and Ifx status in week n+4. The clinical improvement was measured by delta-DAS28 (mean SD) between the patients with or without detectable Ifx levels in the middle of the infusion cycle.

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Contributors ChP, DP-S, EM-M and AB have written this article. DP-S, PA, MTL-C and ChP have carried out the data collection and databases. AV, DP, MGB, PA, AB and ChP have done the clinical evaluation of patients. JD and ChP have done the statistical analysis. DP-S and FA have performed the laboratory assays.

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Competing interests AB has received fees from Roche, Schering-Plough, Wyeth, Abbott, BMS and UCB. E-M-M. is a consultant and a member of the Pfizer speakers bureaus, MSD, UCB and Abbott. ChP, DP-S, MGB and DP have received speaker honoraria from Pfizer. All other authors have declared no conflicts of interest.

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The timing of serum infliximab loss, or the appearance of antibodies to infliximab (ATI), is related with the clinical activity in ATI-positive patients with rheumatoid arthritis treated with infliximab

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ARTICLE 4

TITLE: ***"Predictive Value of Serum Infliximab Levels at Induction Phase in Rheumatoid Arthritis Patients"***

JOURNAL: Submitted in: The Open of Rheumatology Journal

AUTHORS: Jurado Teresa PhD, **Plasencia-Rodríguez Chamaida MD**, Martínez-Feito Ana PhDs, MSc, Navarro-Compán Victoria MD,-PhD, Rispens Theo PhD, de Vries Annick PhD, Bloem Karien PhD, Olariaga Eva-María BSc, Diego Cristina LT, Villalba Alejandro MD, Peiteado Diana MD, Nuño Laura MD, Bonilla Maria-Gema MD, Balsa Alejandro MD,-PhD, Pascual-Salcedo Dora PhD

PATIENTS AND METHODS:

Patients and serum samples

This observational retrospective study included 66 patients with RA recruited from a prospective observational RA-Paz cohort. Patients fulfilled the 1987 revised criteria of the American College of Rheumatology, had moderate or high disease activity (despite previous treatment with disease-modifying anti-rheumatic drugs), and were naïve to treatment with biologics; they were followed-up for at least one year, except when lost to follow-up for medical reasons and sera from most of the visits (at 0-2-6-14-22-54 weeks) had to be available. Patients were given intravenous infusions of 3 mg/kg of Ifx at week (W) 0, 2 and 6 and every 8 weeks thereafter. Serum samples were collected at baseline and immediately before each infusion. Disease activity was assessed at baseline, W22 and W54 using the Disease Activity Score for 28 joints (DAS28) measured according to the erythrocyte sedimentation rate. Therapeutic response was evaluated using the EULAR criteria. Patients whose treatment dose was modified during the time of the study were excluded. The study was approved by La Paz University Hospital Ethics Committee and all patients signed an informed consent form.

Serum Ifx assay

Serum Ifx levels were measured by a capture ELISA as previously described. Coating antibody (MoAb7 anti-TNF) and buffer (High Performance ELISA buffer) were supplied by Sanquin (Amsterdam, The Netherlands). Recombinant TNF was supplied by Preprotech and biotinylated anti-idiotype monoclonal antibody for detection was supplied by Progenika Biopharma (Derio, Spain). Peroxidase-Streptavidin and TMB were used for reaction developing, and optical density was read at 450 nm. The cut-off for positivity was 10ng/ml, established with a control group of 250 sera (150 from healthy donors and 100 from untreated RA patients). Ifx serum levels were evaluated at baseline (W0) and at W2, W6, W14, W22 and W54 after initiation of Ifx treatment. Serum samples from the 6 time points were available from 43 patients, missing a maximum of 2 samples /patient in the rest.

To determine whether the quantitative or qualitative data obtained by our capture ELISA could be extrapolated, we compared our results from University Hospital La Paz (UHLP) with two different commercial ELISAs; Promonitor- Ifx (Progenika Biopharma, Derio, Spain) and Ifx ELISA-Compact produced by Sanquin (Amsterdam, The Netherlands). The Pearson´s correlation coefficients were 0.97 and 0.88, respectively ($p<0.001$ in each comparison), and no discrepancies in positive or negative results were found among all three assays (Kappa correlation index ULPH vs Sanquin =1; Kappa UHLP vs Promonitor=1) (Supplemental Fig 1). The agreement between drug levels of the three different assays was analyzed using an intra-class correlation coefficient (ICC); UHLP vs Sanquin (0.907; 95%IC 0.87-0.93; $p<0.001$) and UHLP vs Promonitor (0.64; 95%IC 0.39-0.78; $p<0.001$). The Bland-Altman plot (Supplemental Fig 2) gave an average of differences with a 95% limit of agreement, indicating that the two assay methods produce similar results.

ATI detection

ATI levels were assessed in sera from W0, W2, W6, W14 and W22 of treatment in 2 different ways:

An in-house 2-site (bridging) ELISA was used to detect uncomplexed (free) ATI as previously described. The cut-off value for the presence of ATI was 50 arbitrary units (AU)/mL, established from the same serum control group as for IFX levels.

A commercial kit called IDKmonitor (Immundiagnostik, Bensheim, Germany) (A), and an acid-dissociation radioimmunoassay (ARIA, Sanquin) (B), were used to measure total (free and complexed) ATI. The (A) assay performed acid dissociation of the serum to acquire free ATI. Peroxidase conjugate-Ifx and biotinylated-Ifx were added to replace the unmarked therapeutic antibody and the marked Ifx formed a complex with ATI that bound via biotin to a streptavidin-coated microtiter plate. Qualitative results higher than 10 AU/mL were considered positive. In the (B) assay, an acid dissociation was also performed to acquire free ATI. Samples were neutralized in a pH 7.6 buffer with an excess of Ifx F (ab´) $_2$-biotin (to replace the unmarked therapeutic antibody). Antibodies were subsequently captured by Protein A Sepharose and radiolabelled streptavidin was used to detect specific ATI, analogue to the adalimumab ARIA.

Because of the retrospective nature of the study not all serum samples were available to be tested in all assays for ATI. Available samples were as follows: W0 (60 by bridging ELISA, 37 by ARIA and 59 by IDKmonitor); W2 (60 by bridging ELISA, 58 by ARIA and 60 by IDKmonitor); W6 (64 by bridging ELISA, 63 by ARIA and 63 by IDKmonitor); W14 (61 by bridging ELISA, 51 by ARIA and 58 by IDKmonitor); W22 (60 by bridging ELISA, 51 by ARIA and 57 by IDKmonitor).

Statistical analysis

Descriptive statistics were reported as mean and standard deviation (SD) or median and interquartile range (IQR) depending on normality. Patients were classified into two groups depending on the presence or absence of circulating serum Ifx at W54. Differences in clinical and biological characteristics between both groups were assessed using Pearson's chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney U test or the Student t-test for continuous variables. Last observation carried forward (LOCF) was employed to impute data for patients who discontinued treatment before the end of the first year.

Serum-dependent ROC curves at W2, W6, W14 and W22 were used to determine the ITL that best predicted the absence of free Ifx at W54. The predictive cut-off was determined as the value for Ifx levels that showed maximum sensitivity, maximum specificity and higher positive likelihood ratio at each studied time point. Ifx treatment survival was studied using Kaplan-Meier curves and groups were compared using the log-rank test.

Univariable and multivariable logistic regression models were employed to investigate the association between early-stage ITL and Ifx at W54. Demographic, clinical and serological characteristics (including ITL at W2 and W6 as categorical variables) were included in the models as independent variables and the Ifx levels as the dependent variable. For all the analysis, Graph Pad Prism 6 (San Diego, CA, USA) and SPSS 21.0 software were employed and p-values <0.05 were considered statistically significant.

TITTLE PAGE

PREDICTIVE VALUE OF SERUM INFLIXIMAB LEVELS AT INDUCTION PHASE IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Aims: To analyze whether serum infliximab (IFX) trough levels (ITL) at the induction phase are associated with IFX clearance and clinical outcomes at week (W) 54 and to investigate the association with immunogenicity development in rheumatoid arthritis (RA).

Methods: Observational retrospective study in which ITL from 66 RA patients were measured by capture ELISA at W0, W2, W6, W14 and W22. Patients were classified as ITLpos if IFX was detectable at W54 and ITLneg otherwise. Antibodies towards IFX (ATI) were assayed by bridging ELISA and by two drug-tolerant assays. ITL cut-off values were established by ROC curves. The association between ITL at early-stage and clearance of IFX at W54 was analyzed by univariable and multivariable logistic regression.

Results: At all time points, ITLneg patients (n=25) had significantly lower levels than ITLpos (n=41). An ITL cut-off value of 4.4 µg/mL at W6 best predicted W54 IFX absence. In the univariable analysis, 3 factors were associated with IFX absence at W54: having an ITL below the cut-off at W2 or W6, non-use of methotrexate and an older age. In the multivariable analysis, only ITL below the cut-off at W6 (OR: 86.6; 95%CI: 6.58-1139.99) and non-use of methotrexate (OR: 6.9; 95%CI: 1.04-45.84) remained significantly associated with W54 IFX absence. The ATI presence was more frequent in patients with ITL below the cut off at early weeks.

Conclusions: In RA, early-stage ITL are inversely associated with IFX clearance, early treatment discontinuation and clinical outcomes at W54. ATI was the main reason for low early ITL. A W6 cut-off value of 4.4 µg/mL is proposed as an useful prognostic measure of treatment efficacy.

PREDICTIVE VALUE OF SERUM INFLIXIMAB LEVELS AT INDUCTION PHASE IN RHEUMATOID ARTHRITIS PATIENTS

BACKGROUND

Infliximab (IFX), a chimeric anti-tumor necrosis factor monoclonal antibody, is effective in the treatment of rheumatoid arthritis (RA) and other inflammatory diseases (1-4). However, treatment fails in around 40% of patients, in many cases due to immunogenicity. An important contributor to treatment failure is the presence of antibodies towards IFX (ATI). The formation of immune complexes between antibodies and biological drugs may increase drug clearance, reduce serum levels and result in a loss of efficacy (5-8).

Several publications have reported an association between serum IFX levels and clinical response (5;6;9;10). A good clinical response is usually associated with high circulating IFX levels (11;12). On the other hand, low or undetectable circulating IFX levels may indicate ATI development with the consequent loss of drug efficacy.

Most of the methods available to detect ATI are hindered by the simultaneous presence of drug and antibodies in serum, being the bridging enzyme-linked immunosorbent assay (ELISA) the most susceptible one to this effect (13). Drug interference accounts for important discrepancies between studies, so current efforts are focused on the development of assays lacking drug interference that can reveal the true prevalence of immunogenicity.

The association between early-stage IFX serum concentrations, clinical outcomes and immunogenicity has recently been studied for inflammatory diseases such as RA and ulcerative colitis (UC) (12;14). It is not clear why some patients show faster drug clearance from treatment commencement, being the hypothesis that circulating drug levels are affected by patient characteristics associated with baseline disease activity and an early anti-drug antibody production (15;16).

The aim of this study is to analyze whether serum IFX trough levels (ITL) at the induction phase are associated with IFX clearance and clinical outcomes in the first year of treatment.
The implication of different factors potentially accounting for the presence of low levels at early stages of treatment is also studied.

PATIENTS AND METHODS

Patients and serum samples

This observational retrospective study included 66 patients with RA recruited from a prospective observational cohort of patients treated with biological drugs at the rheumatology department of La Paz University Hospital (Madrid). Patients fulfilled the 1987 revised criteria of the American College of Rheumatology (17), had moderate or high disease activity (18) (despite previous treatment with disease-modifying anti-rheumatic drugs), and were naïve to treatment with biologics; they were followed-up for at least one year, except when lost to follow-up for medical reasons and sera from most of the visits (at 0-2-6-14-22-54 weeks) had to be available. Patients were given

intravenous infusions of 3 mg/kg of IFX at week (W) 0, 2 and 6 and every 8 weeks thereafter. Serum samples were collected at baseline and immediately before each infusion. Disease activity was assessed at baseline, W22 and W54 using the Disease Activity Score for 28 joints (DAS28) measured according to the erythrocyte sedimentation rate. Therapeutic response was evaluated using the criteria of the European League Against Rheumatism (EULAR) (19). Patients whose treatment dose was modified during the time of the study were excluded.

The study was approved by La Paz University Hospital Ethics Committee and all patients signed an informed consent form.

Serum IFX assay

Serum IFX levels were measured by a capture ELISA as previously described (5). Coating antibody (MoAb7 anti-TNF) and buffer (High Performance ELISA buffer) were supplied by Sanquin (Amsterdam, The Netherlands). Recombinant TNF was supplied by Preprotech and biotinylated anti-idiotype monoclonal antibody for detection was supplied by Progenika Biopharma (Derio, Spain). Peroxidase-Streptavidin and TMB were used for reaction developing, and optical density was read at 450 nm. The cut-off for positivity was 10ng/ml, established with a control group of 250 sera (150 from healthy donors and 100 from untreated RA patients). IFX serum levels were evaluated at baseline (W0) and at W2, W6, W14, W22 and W54 after initiation of IFX treatment. Serum samples from the 6 time points were available from 43 patients, missing a maximum of 2 samples /patient in the rest.

To determine whether the quantitative or qualitative data obtained by our capture ELISA could be extrapolated, we compared our results from University Hospital La Paz (UHLP) with two different commercial ELISAs; Pomonitor-IFX (Progenika Biopharma, Derio, Spain) and IFX ELISA-Compact produced by Sanquin (Amsterdam, The Netherlands). The Pearson´s correlation coefficients were 0.97 and 0.88, respectively ($p<0.001$ in each comparison), and no discrepancies in positive or negative results were found among all three assays (Kappa correlation index ULPH vs Sanquin =1; Kappa UHLP vs Promonitor=1) (Supplemental Fig 1). The agreement between drug levels of the three different assays was analyzed using an intra-class correlation coefficient (ICC); UHLP vs Sanquin (0.907; 95%IC 0.87-0.93; $p<0.001$) and UHLP vs Promonitor (0.64; 95%IC 0.39-0.78; $p<0.001$). The Bland-Altman plot (Supplemental Fig 2) gave an average of differences with a 95% limit of agreement, indicating that the two assay methods produce similar results.

ATI detection

ATI levels were assessed in sera from W0, W2, W6, W14 and W22 of treatment in 2 different ways:

1. An in-house 2-site (bridging) ELISA was used to detect uncomplexed (free) ATI as previously described (5). The cut-off value for the presence of ATI was 50 arbitrary units (AU)/mL, established from the same serum control group as for IFX levels.
2. A commercial kit called IDKmonitor (Immundiagnostik, Bensheim, Germany) (A), and an acid-dissociation radioimmunoassay (ARIA, Sanquin) (B), were used to measure total (free and complexed) ATI. The (A) assay performed acid dissociation of the serum to acquire free ATI. Peroxidase conjugate-IFX and biotinylated-IFX were added to replace the unmarked therapeutic antibody and the marked IFX formed a complex with ATI that bound via biotin to a streptavidin-coated microtiter plate. Qualitative results higher than 10 AU/mL were considered positive. In the (B) assay, an acid dissociation was also

performed to acquire free ATI. Samples were neutralized in a pH 7.6 buffer (20) with an excess of IFX $F(ab')_2$-biotin (to replace the unmarked therapeutic antibody). Antibodies were subsequently captured by Protein A Sepharose and radiolabelled streptavidin was used to detect specific ATI, analogue to the adalimumab ARIA (20).

Because of the retrospective nature of the study not all serum samples were available to be tested in all assays for ATI. Available samples were as follows: W0 (60 by brigding ELISA, 37 by ARIA and 59 by IDKmonitor); W2 (60 by brigding ELISA, 58 by ARIA and 60 by IDKmonitor); W6 (64 by brigding ELISA, 63 by ARIA and 63 by IDKmonitor); W14 (61 by brigding ELISA, 51 by ARIA and 58 by IDKmonitor); W22 (60 by brigding ELISA, 51 by ARIA and 57 by IDKmonitor).

Statistical analysis

Descriptive statistics were reported as mean and standard deviation (SD) or median and interquartile range (IQR) depending on normality. Patients were classified into two groups depending on the presence or absence of circulating serum IFX at W54. Differences in clinical and biological characteristics between both groups were assessed using Pearson's chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney U test or the Student t-test for continuous variables. Last observation carried forward (LOCF) was employed to impute data for patients who discontinued treatment before the end of the first year.

Serum-dependent receiver operating characteristic (ROC) curves at W2, W6, W14 and W22 were used to determine the ITL that best predicted the absence of free IFX at W54. The predictive cut-off was determined as the value for IFX levels that showed maximum sensitivity, maximum specificity and higher positive likelihood ratio at each studied time point. IFX treatment survival was studied using Kaplan-Meier curves and groups were compared using the log-rank test.

Univariable and multivariable logistic regression models were employed to investigate the association between early-stage ITL and IFX at W54. Demographic, clinical and serological characteristics (including ITL at W2 and W6 as categorical variables) were included in the models as independent variables and the IFX levels as the dependent variable.

For all the analysis, GraphPad Prism 6 (San Diego, CA, USA) and SPSS 21.0 software were employed and p-values <0.05 were considered statistically significant.

RESULTS

1. Patient characteristics

Baseline characteristics for the 66 patients with RA starting IFX are summarized in Table 1. Most patients were female (86%) and were positive for both rheumatoid factor (82%) and anti–cyclic citrullinated peptide antibody (82%). At inclusion time, all patients had active disease (DAS28: 5.5±1.3). Median disease duration prior to IFX treatment was 14 years (IQR: 9-18). Out of the 66 patients, 42 (64%) received concomitant treatment with

methotrexate (MTX, mean dose 15 mg/week). Eight out of 66 (12%) patients discontinued the IFX treatment before the first year (5 due to lack of efficacy and 3 due to adverse events).

Patients were divided into 2 groups depending on the presence (ITLpos) or absence (ITLneg) of circulating serum IFX at W54 (Table 1). At W54, ITL were undetectable in 25 patients (38%), 23 of whom were ITLneg already since W22. ITLpos patients had lower baseline DAS28 scores compared to ITLneg patients (5.3±1.1 vs 5.8±1.2; p=0.06) and their mean (SD) IFX levels at W54 were 2.1 (1.8) µg/mL. Less ITLneg patients were treated with methotrexate (MTX) (11 (26%)) than ITLpos patients (32 (74%)); p≤0.01. ITLpos patients were older than ITLneg patients (59 years (51-69) vs 51 years (42-65)); p≤0.05. And the baseline C-reactive protein (CRP) was higher in ITLneg patients than in ITLpos patients (23±2 vs 15±17; p≤0.05).

2. Association between early-stage ITL and W54 IFX status

The ITLneg patients at W54 had lower early-stage ITL than the ITLpos patients (W2: 20.0 ±12.7 µg/mL vs 29.7±14.5 µg/mL (p=0.015); W6: 4.2±5.9 µg/mL vs 15.7±11.1 µg/mL (p<0.0001); W14: 0.1±0.2 µg/mL vs 4.1±5.3 µg/mL (p<0.0001); and W22: 0.01±0.04 µg/mL vs 2.8±3.3 µg/mL (p<0.0001)) (Fig 1A). At all studied time points, the areas under the curve (AUC) of the ROC curves were statistically different from 0.5 (Fig. 1B), enabling cut-off levels to distinguish between ITLpos and ITLneg patients at W54 (Table 2). Out of the 25 ITLneg patients at W54, 12 (48%), 18 (72%) and 25 (100%) had ITL below the predictive cut-off at W2, W6 and W14, respectively. At W14, in 19 out of 25 patients (76%) ITL were undetectable. The W6 predictive cut-off (4.4 µg/mL) showed the best association with IFX absence at W54, with a sensitivity of 70% (95% confidence interval (CI): 45.7-88.1), a specificity of 95% (95% CI: 83.1-99.4) and a positive likelihood ratio of 14. Therefore, the W6 predictive cut-off was considered the reference value to predict clinical and serological outcomes for further analysis.

3. Analysis of association between early-stage ITL and other confounder factors with W54 IFX status

In the univariable analysis, three factors were significantly associated with IFX absence at W54 (Table 3a): i) having an ITL below the cut-off at W2 (odds ratio (OR): 12.40; 95% CI: 3.48-44.15) or at W6 (OR: 44.33; 95% CI: 7.99-246.03), ii) non-use of MTX (OR: 4.20; 95% CI: 1.33-13.32) and iii) being older (OR: 1.04; 95% CI: 1.03-1.07).

In the multivariable logistic regression analysis, even after adjusting for possible confounders (age, gender and baseline DAS28), the ITL below the cut-off at W2 (OR: 15.85; 95% CI: 2.95-85.03; p=0.01) or at W6 (OR: 86.64; 95% CI: 6.58-1139.99) and also the non-use of MTX (OR: 12.26; 95% CI: 1.83-82.22) remained significantly associated with IFX absence at W54 (Table 3b).

4. Association between early-stage ITL (W6) and clinical outcomes (W54)

Patients with ITL above the W6 predictive cut-off (4.4 µg/ml) had lower DAS28 scores at W54 than patients with ITL below the cut-off (3.68±1.26 vs 4.75±1.27; p=0.01) (Fig 2A). Most patients with low disease activity or remission by DAS28 at W54 had ITL above predictive cut-off at W6 (ITL above: 20 of 45 patients (44%) vs ITL below: 3 of 19 patients (16%); p=0.02) (Fig. 2B). Likewise, most EULAR responders at W54 had ITL above the

predictive cut-off at W6, namely 33 (73%) of 45 patients were EULAR responders with ITL above the cut-off vs 10 out of 19 EULAR responders (53%) with ITL below the cut-off; p=0.08.

5. Association between IFX survival and early-stage predictive cut-off

Patients with ITL below the predictive cut-off at any of the studied time points dropped out of IFX treatment earlier (for patients with ITL above vs below: at W2, 4 years (1.1-3.6) vs 2 years (0.3-0.9), p=0.01; at W6, 5 years (1.6-5.0) vs 1.7 years (0.2-0.6), p=0.01; and at W14, 6.3 years (1.5-4.9) vs 2.3 years (1.1-3.8), p=0.03) (see W6 data in Fig 2C).

6. Association between early-stage ATI detection and W54 IFX status

Most patients with ATI had ITL below the cut-off at W6 (Table 4). The IDKmonitor and ARIA drug-tolerant assays detected ATI production earlier than bridging ELISA, which only detected ATI if not complexed to IFX (Table 4). At baseline time, no patient had detectable ATI by any assayed methods. At W54, all the ITLneg patients were ATI-positive according to bridging ELISA.

DISCUSSION

We have demonstrated that low ITL during induction phase are associated with IFX clearance and poor clinical outcomes after the first year of treatment in patients with RA. On top of ITL at W2 and especially at W6, non-use of MTX has also been observed to be predictive of IFX absence at W54. Furthermore, the early development of immunogenicity was found to be correlated with low ITL.

Some studies of patients with RA have reported an association between early IFX concentrations, drug maintenance throughout treatment and the detection of immunogenicity (12;21). Ducourau *et al.,* (12) showed that low IFX concentrations from W2 to W14 were associated with increased ATI development and lower drug survival in patients with RA and patients with spondyloarthritis. Bendtzen *et al.,* (21) reported that low IFX levels 6 weeks after starting therapy were predictive of ATI detection and the consequent loss of circulating IFX at 6 months of treatment in patients with RA. From our cohort, we could confirm that patients with low early-stage ITL were more prone to circulating drug loss after 1 year of treatment. Based on the results, this is the first time where a cut-off value of 4.4 µg/mL for ITL at W6 is defined as a predictor of efficacy and drug survival at W54 in RA patients.

Although low ITL during treatment have been reported as being associated with therapy failure in several publications (5;11;22), there are only limited data available for the association between early-stage ITL and clinical response throughout treatment (11;14;23). Mulleman *et al.,* (11) described an association between IFX concentrations during the first months of treatment and clinical response. Kobayashi *et al.,* (14) reported that on the basis of a controlled trial of 208 patients with UC, ITL at W2 above 21.3 µg/mL were significantly associated with both 14-week remission and 30-week mucosa healing. They also postulated that early-stage ITL could be used to predict treatment outcomes in UC patients. Van den Bemt *et al.,* (23) showed from a cohort of 57 patients with RA that ITL at W6 together with disease activity scores optimized early detection of non-responders to IFX therapy. Other studies including patients with psoriasis and RA monitored adalimumab and etanercept levels at treatment

CONCLUSION

In conclusion, to our knowledge, this is the first study with RA patients that establishes an association between early-stage ITL (at W2 and W6) and IFX loss, early treatment dropout and clinical outcomes after the first year of treatment. Furthermore, it also suggests that ATI development is the main reason for low early-stage circulating IFX levels. Finally, a cut-off value for ITL at W6 is also proposed. This cut-off value could provide clinicians a useful tool to predict treatment efficacy in patients with RA treated with IFX.

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Author contributions: TJ, CP-R and DP-S were involved in the study design, the patients´ selection and data collection. TJ, DP-S, CP-R and AM-F wrote the article. AB, VN-C, KB and TR reviewed the article. CP-R, AV, M-GB, LN and DP and were directly involved in patient management. AM-F, EO, CD, AV and KB performed the serum assays. All authors read and approved the final manuscript.

Figures:

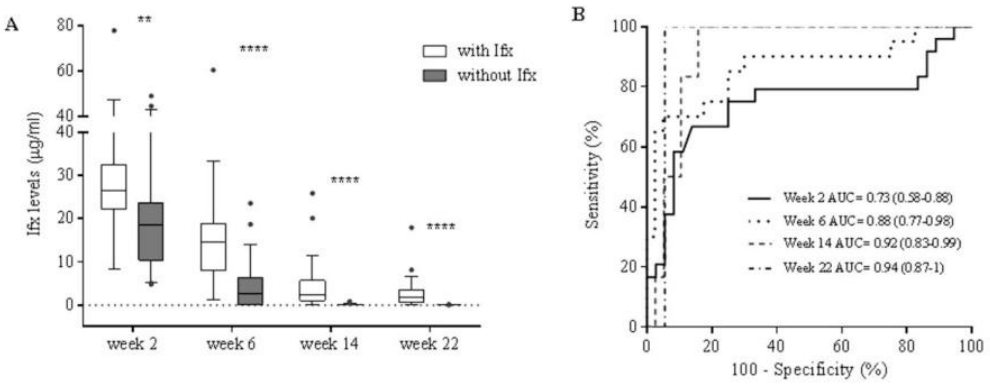

Fig 1: A) Infliximab (Ifx) trough levels (ITL) in patients with rheumatoid arthritis at early treatment stages, according to week (W) 54 Ifx status. Results are shown as medians (solid lines within boxes), interquartile ranges (upper and lower box boundaries) and maximums and minimums. Black dots indicate outliers. B) Receiver operating characteristic curves for ITL at W2, W6, W14 and W22 for prediction of Ifx absence at W54. AUC, Area Under the Curve.

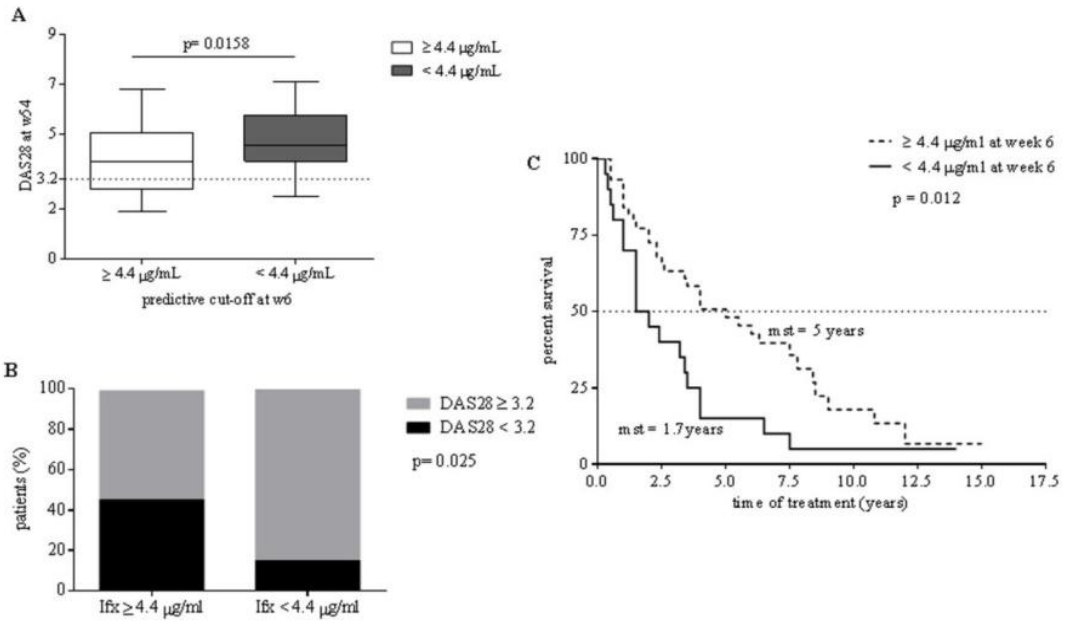

Fig 2: A) Disease Activity Score in 28 joints (DAS28) (median and interquartile ranges) for patients with rheumatoid arthritis (RA) with infliximab (Ifx) trough levels (ITL) above and below the week 6 predictive cut-off. B) Patients (%) with low disease activity or remission (DAS28 < 3.2) at W54, according to the week 6 ITL cut-off. C) Kaplan-Meier curves for Ifx treatment survival, according to the week 6 predictive cut-off. Differences between groups were evaluated by the log- rank test. Mean survival time (mst).

Tables

Table 1: Demographic characteristics of 66 patients with rheumatoid arthritis (RA).

Characteristics	Patients (n=66)	ITLpos at week 54 (n=41)	ITLneg at week 54 (n=25)	p
Age, years*	56 (47-68)	59 (51 69)	51 (42-65)	<0.05
Body mass index*	24 (22-27)	24 (22-27)	23(20-28)	0.2
Female**	57 (86 %)	35 (85%)	22 (88%)	1
Disease duration, years*	14 (9-18)	16 (10-19)	12 (5-16)	0.1
Rheumatoid factor**	54 (82%)	33(80%)	22(96.6%)	0.3
ACPA**	54 (82%)	32(82%)	22(88%)	0.2
DAS28 at baseline***	5.5 (1.3)	5.3 (1.1)	5.8 (1.4)	0.06
CRP at baseline***	18 (19)	15 (17)	23 (20)	0.05
Concomitant treatment:				
Methotrexate**	43 (65%)	32 (74%)	11 (26%)	<0.01
Methotrexate dose, mg/week*	15.6 (5.5)	12.2 (8.5)	8.8 (7.4)	0.1
Others DMARDs **	16 (24%)	7 (17%)	9 (36%)	0.6
Prednisone**	43 (65%)	31 (75%)	12 (48%)	<0.05

*Median (interquartile range); ** n (%); *** mean (standard deviation)
Body mass index(kg/m^2); ACPA, anti-citrullinated protein antibodies(IU/ml); CRP, C-reactive protein(mg/L); DAS28, Disease Activity Score in 28 joints; DMARD, disease-modifying anti-rheumatic drug; IL-6

Table 2. Early-stage treatment Infliximab level cut-offs with sensitivity, specificity and positive likelihood ratio (LR+) values.

Week	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	LR+
2	21.2 μg/mL	67% (44-84)	86% (70-95)	4.8
6	4.4 μg/mL	70% (45-88)	95% (83-99)	14
14	0.4 μg/mL	83% (35-99)	89% (75-97)	7.9
22	0.2 μg/mL	100% (15-100)	94% (81-99)	18

CI, confidence interval; LR, Likelihood Ratio.

Table 3. Univariable and multivariable logistic regression analyses for predicting ITL absence at week (W) 54.

a. Univariable analyses for clinical baseline factors and early-stage infliximab (IFX) trough levels (ITL) for ITL absence at week (W) 54.

Factors	OR	95%CI
At baseline		
Female sex	1.59	0.29-8.69
Age	1.04	1.03-1.07
Rheumatoid factor	0.54	0.13-2.18
ACPA	0.21	0.24-1.81
Body mass index	1.05	0.93-1.18
DAS28	0.70	0.46-1.05
MTX non-use	4.20	1.33-13.32
CRP levels	0.98	0.95-1.05
Levels below cut-off at W2 (21.2 µg/mL)	12.40	3.48-44.15
Levels below cut-off at W6 (4.4 µg/mL)	44.33	7.99-246.03

b. Multivariable logistic regression analysis for IFX absence at W54 including ITL at W2 (Model I) and at W6 (Model II) as possible predictors.

Factors	OR	95%CI
Model I		
Female sex	1.09	0.09-13.18
Age	1.09	1.03-1.17
DAS28	0.64	0.36-1.14
MTX non-use	12.26	1.83-82.22
Levels below cut-off at W2 (21.2 µg/mL)	15.85	2.95-85.03
Model II		
Female sex	0.65	0.05-7.97
Age	1.05	0.98-1.12
DAS28	0.64	0.31-1.30
MTX non-use	6.91	1.04-45.84
Levels below cut-off at W6 (4.4 µg/mL)	86.64	6.58-1139.99

ACPA, anti-cyclic citrullinated peptide antibodies; CRP, C-reactive protein; CI, confidence interval; DAS28, Disease Activity Score in 28 joints; IL-6, interleukin-6; MTX, methotrexate; OR, odds ratio; TNF-α, tumour necrosis factor α; ITL, Infliximab trough levels.

Table 4. ATI frequency assayed by three different methods for all studied time points.

	(27) µg/mL at W6	bridging ELISA	IDKmonitor	ARIA
W2	IFX ≥4.4 n=44	0	0	0
	IFX <4.4 n=20	0	1 (5%)	1 (5%)
	p	1	0.33	0.33
W6	IFX ≥4.4 n=44	0	1 (2.3%)	2 (4.5%)
	IFX <4.4 n=20	4 (20%)	7 (35%)	10 (50%)
	p	0.007	0.002	0.001
W14	IFX ≥4.4 n=44	2 (4.5%)	7 (16%)	12 (27.3%)
	IFX <4.4 n=20	11 (55%)	16 (80%)	16 (80%)
	p	<0.0001	<0.0001	0.0006
W22	IFX ≥4.4 n=44	5 (11.4%)	20 (45.5%)	25 (56.8%)
	IFX <4.4 n=20	15 (75%)	18 (90%)	18 (90%)
	p	<0.0001	<0.0001	0.01

ARIA, acid-dissociation radioimmunoassay (Sanquin, Amsterdam, The Netherlands); IDKmonitor, commercial kit (Immundiagnostik, Bensheim, Germany); IFX, infliximab;
W, week.

Supplemental Figures.

Supplemental Fig. 1: Correlation between UHLP capture ELISA and two different ELISAs (PROMONITOR and SANQUIN). A) Infliximab (IFX) trough levels (ITL) in patients with rheumatoid arthritis (n=124) measured by UHLP capture ELISA and SANQUIN ELISA. Results are shown correlations between both assays. **B)** Infliximab (IFX) trough levels (ITL) in patients with rheumatoid arthritis (n=77) measured by UHLP capture ELISA and PROMONITOR ELISA. Results are shown correlations between both assays.

Supplemental Fig. 2: Comparison of IFX methods by Bland-Altman analysis. A) Bland-Altman analysis between UHLP and SANQUIN, the differences between the two measurements (Y-axis in µg/ml) is plotted against the average of each pair of measurements (X-axis in µg/ml), the 95% limits of agreement are represented by dot line. **B)** Bland-Altman analysis between UHLP and Promonitor, the differences between the two measurements (Y-axis in µg/ml) is plotted against the average of each pair of measurements (X-axis in µg/ml), the 95% limits of agreement are represented by dot line.

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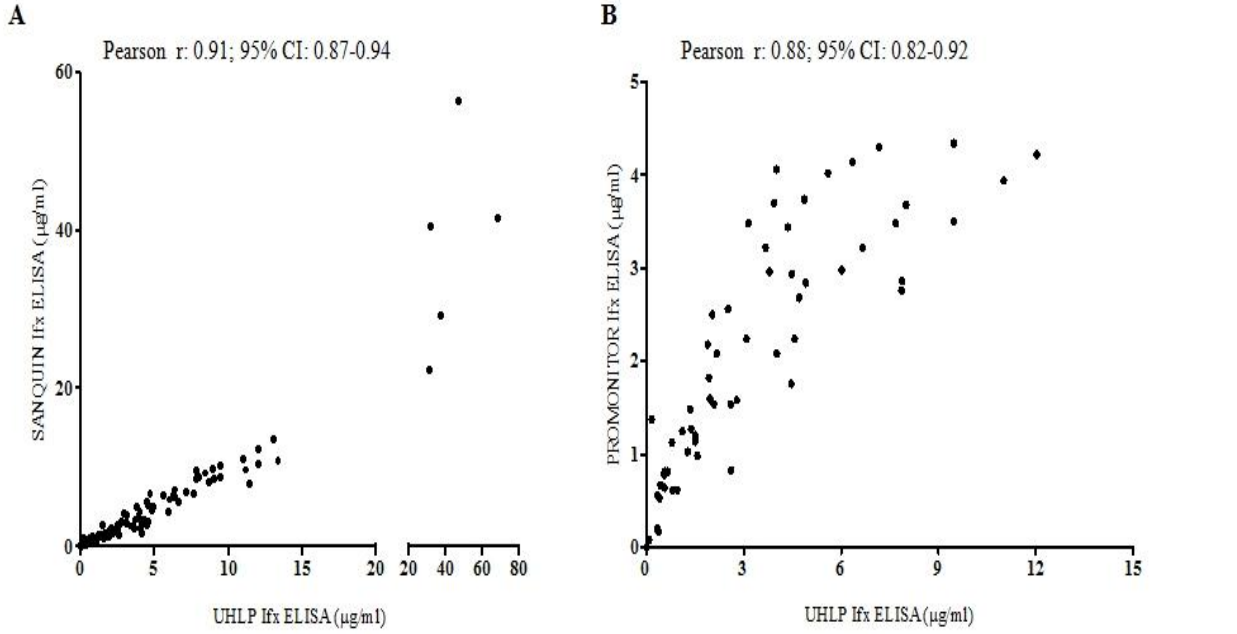
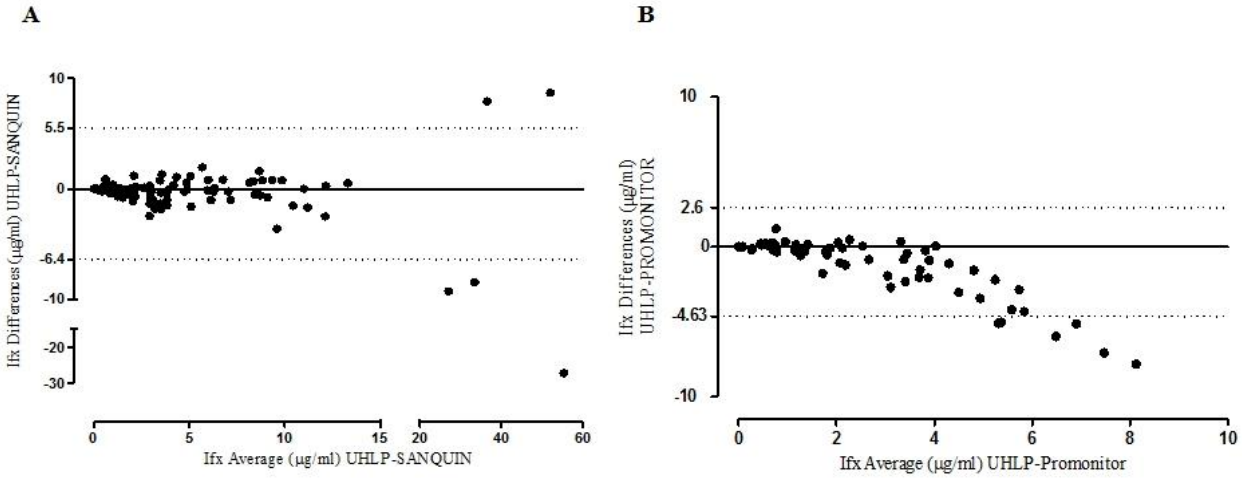

A
Pearson r: 0.91; 95% CI: 0.87-0.94
SANQUIN Ifx ELISA (µg/ml)
UHLP Ifx ELISA (µg/ml)
B
Pearson r: 0.88; 95% CI: 0.82-0.92
PROMONITOR Ifx ELISA (µg/ml)
UHLP Ifx ELISA (µg/ml)


A
Ifx Differences (µg/ml) UHLP-SANQUIN
5.5
-6.4
Ifx Average (µg/ml) UHLP-SANQUIN
B
Ifx Differences (µg/ml) UHLP-PROMONITOR
2.6
-4.63
Ifx Average (µg/ml) UHLP-Promonitor

ARTICLE 5

TITLE: ***"Serum tocilizumab trough concentration can be used to monitor systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study"***

JOURNAL: Scand J Rheumatol 2016;0(0):1–8(85)(85)

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PATIENTS AND METHODS:

Patient population and study design

This prospective observational cohort consisted of 66 consecutively included adult RA patients diagnosed according to the American College of Rheumatology

(ACR) 1987 revised criteria. All patients started Tcz between April 2009 and June 2014. Two cohorts were combined (The Netherlands, n = 34; Spain, n = 32). All patients had active disease (DAS28-ESR > 3.2), despite prior treatment with disease-modifying anti-rheumatic drugs (DMARDs) and/or biologics. All patients started with the Tcz standard regimen (8 mg/kg every 4 weeks iv) and concomitant DMARDs with or without prednisone, only with concomitant prednisone or TCZ as monotherapy. Adaptations in the Tcz regimen could be made, based on the expert opinion of the rheumatologist. Patients were eligible for inclusion in the final analyses if a serum sample (trough concentration) from at least one follow-up visit from week 12 onwards was available, taken after the Tcz standard regimen and in combination with the availability of corresponding measurements of DAS28 and/or CRP. The study was approved by the Medical Ethics Committee of Slotervaart Hospital and the Jan van Breemen Research Institute/Reade, Amsterdam, The Netherlands, and by the Medical Ethics Committee of La Paz Hospital, Madrid, Spain.

Outcome measures

In the Dutch cohort, DAS28, parameters of inflammation (CRP and ESR), and serum trough samples were collected at baseline and 4, 12, and 24 weeks thereafter. In the Spanish cohort, serum samples and parameters of inflammation were collected before every Tcz infusion whereas DAS28 and its separate components were measured at baseline and at 24 weeks. The duration of follow-up in this study was 24 weeks.

To investigate the relationship between serum Tcz trough concentration (described in the following as Tcz concentration) and clinical response at week 24, defined as an improvement compared to baseline in DAS28 (ΔDAS28) and swollen joint count in 28 joints (ΔSJC28). To obtain the concentration–effect curve at week 24, the last observation carried forward (LOCF) method was used for patients in whom follow-up data from week 12 were available but not yet those from week 24. The relationship between Tcz concentration and serum CRP (described in the following as CRP) was investigated separately as CRP can be used as a surrogate marker for systemic IL-6R blockade.

TCZ concentration measurement

To measure Tcz concentrations an immunoassay was developed using rabbit ADA to capture Tcz, and rabbit anti- Tcz F (ab′) 2 fragments for detection. Maxisorp enzyme-linked immunosorbent assay (ELISA) plates were coated overnight at room temperature with 0.125 μg/mL rabbit anti-Tcz in phosphate-buffered saline (PBS). The specific rabbit anti-idiotype antibodies were produced analogously as described for natalizumab. Plates were washed five times with PBS/0.02% Tween (PT), then washed and incubated for 1 h with patient serum that had been serially diluted in high-performance ELISA (HPE) buffer. After washing five times with PT, plates were incubated for 1 h with biotinylated anti-Tcz F (ab′) 2 fragments (125 nag/mL in HPE buffer). After washing, streptavidin–poly-horseradish peroxidase (poly-HRP); Sanquin, Amsterdam, The Netherlands) (1:10 000, in HPE buffer) was added for 1 h at 37°C. After washing, the ELISA was developed with 100 μg/mL tetramethylbenzidine in 0.11Msodium acetate (pH 5.5) containing 0.003% (v/v) H2O2. The reaction was

stopped with 2 M H2SO4. Absorption was measured at 450 nm relative to a titration curve of Tcz in each plate. The lower limit of quantification (LLOQ) in serum was 200 nag/mL; the overall precision and accuracy were 8% and 93%, respectively. Serum samples were collected at the trough concentrations, that is just before the next infusion.

Anti-Tcz antibody measurement

Measurement of anti-Tcz antibodies was essentially carried out as described previously. One microlitre of serum diluted in buffer containing intravenous immunoglobulin (IVIg) F(ab′)2 to prevent ant hinge reactivity was incubated overnight with 1 mg Protein A Sepharose (GE Healthcare, Chalfont St Giles, UK) and 2.5 nag biotinylated F(ab′)2 Tcz in a final volume of 800 µL. Subsequently, samples were washed with 0.005% PT and about 1 nag 125I-labelled streptavidin was added in a 800-µL final volume of PBS albumin Tween [PBS/0.01 M ethylene demine- tetra acetic acid (EDTA)/0.3% bovine serum albumin/0.004% Tween- 20/0.05% NaN3] and incubated overnight. Unbound label was removed by washing and Sepharose-bound radioactivity was measured. Antibody levels were compared to a standard serum sample of an immunized rabbit containing ADA and expressed in arbitrary units (AU). A lower limit of detection was based on mean +3 standard deviations (SD) measured in a panel of 50 sera from healthy donors and 15 sera containing ACPA, antinuclear antibodies (ANA), and/or RF.

Statistical analyses

For statistical analyses, SPSS version 21.0 and Graph Pad Prism 6.0 for Windows were used. The results are displayed as number and percentage or mean ± SD when normally distributed, or as median and interquartile range (IQR) when non-normally distributed. For differences in baseline characteristics between patients of the Spanish cohort vs. the Dutch, an independent-sample t-test, the Mann–Whitney U test, or the $\chi 2$ test was used, as appropriate. The threshold for significance was set at $p < 0.05$. Spearman's rank test was used to investigate the correlation between Tcz concentration and CRP. A linear regression analysis was used to investigate the

relationship between Tcz concentration and ΔDAS28 at week 24. Potential confounders of this relationship were investigated using the baseline characteristics, excluding the separate components of the DAS28, as the baseline DAS28 was already included. A variable was considered to be a confounder if it changed the regression coefficient by ≥ 10%. For this analysis, the LOCF method was used as described earlier. Sensitivity analyses showed no significant difference in baseline characteristics or outcome data (ΔDAS28 and Tcz concentrations) at week 12 between patients with and without outcome data at week 24.

Serum tocilizumab trough concentration can be used to monitor systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study

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Objectives: To investigate the pharmacokinetics (PK) and dynamics of tocilizumab (TCZ) in daily practice.
Method: An observational study of 66 consecutive RA patients treated with TCZ 8 mg/kg once every 4 weeks intravenously, monitored for 24 weeks. Spearman s rank test was used to investigate the correlation between TCZ

Score in 28 joints (DAS28) compared to baseline, and its relationship with TCZ concentration was investigated using linear regression analyses. TCZ trough concentrations and anti-drug antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) and antigen binding test, respectively.
Results: At baseline, 26 patients (39.4%) had a CRP level above 10 mg/L with a median (interquartile range, IQR) of 37.7 (21.9 49.7) mg/L. A TCZ concentration above 1 mg/L was sufficient to normalize CRP levels. Spearman s rank test showed a correlation coefficient of –0.460 ($p < 0.0001$). The TCZ concentration varied widely, with concentrations < 1 mg/L in 17
one sample. Linear regression analyses showed a coefficient of 0.080 with a 95% confidence interval (CI) of 0.039 0.113 ($p < 0.001$) for the association between TCZ concentration and ΔDAS28. No confounders were identified.
Conclusions: The TCZ standard regimen results in a wide variety of serum TCZ trough concentrations; this is mostly due to target binding and to a lesser extent to immunogenicity. The majority of patients obtained TCZ concentrations > 1 mg/L, which is sufficient for CRP normalization. Therefore, dose taper strategies might be possible in a substantial proportion of patients.

The pathogenesis of rheumatoid arthritis (RA) involves flammatory mediators such as interleukin (IL)-6. IL-6 is a multifunctional cytokine and is associated with inflammation, chronic synovitis (1, 2), bone destruction of joints (3), and the pathogenesis of RA (4, 5). Moreover, IL-6 is the most important cytokine-stimulating hepatocyte to produce C-reactive protein (CRP) (1, 6, 7). IL-6 can activate target cells, such

occurs in the body as a membrane-bound (mIL-6R) and a soluble form (sIL-6R); the activation takes place though classic- and trans-signalling pathways, respectively. The sIL-6R/IL-6 complex can only activate cells that express cell-surface glycoprotein-130 (8).

Tocilizumab (TCZ) is a humanized antibody that competitively inhibits both sIL-6R and mIL-6R and is an effective treatment for RA (9, 10). Currently, TCZ can be administered intravenously (iv) or subcutaneously (11), with or without concomitant methotrexate (10). Randomized controlled trials (RCTs) have shown that TCZ treatment substantially reduces biomarkers of inflammation, such as CRP, and influences serum levels of IL-6 and sIL-6R (10 14). With regard to CRP, Nishimoto et al have shown that a TCZ concentration above 1 mg/L is sufficient for CRP normalization (12). Although clinical response rates are promising, not all patients seem to respond adequately to TCZ treatment, as has also been reported for anti-tumour necrosis factor (TNF) treatment (15).

Clinical inefficacy with regard to biological treatment is

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may be a major factor influencing the pharmacokinetics (PK). The presence of high titres of anti-drug antibodies (ADA) reduces the amount of free drug available to bind the target, resulting in a reduced clinical response in the majority of patients with detectable ADA (16). Evolving evidence shows that the PK of TCZ is influenced by target binding (amount of IL-6R) and to a lesser extent by immunogenicity (10, 14, 17).

The identification of factors that can predict the clinical response to a biological agent is important because this knowledge can be used to optimize treatment in individual patients. Currently, all data on serum TCZ concentrations, immunogenicity, and clinical response are obtained from RCTs. The aim of this study was to investigate variation in serum TCZ trough concentrations and the relationship with clinical measurements in RA during 24 weeks of follow-up.

Method

Patient population and study design

This prospective observational cohort consisted of 66 consecutively included adult RA patients diagnosed according to the American College of Rheumatology (ACR) 1987 revised criteria (18). All patients started TCZ between April 2009 and June 2014. Two cohorts were combined (The Netherlands, n = 34; Spain, n = 32). All patients had active disease, that is a Disease Activity Score in 28 joint counts using the erythrocyte sedimentation rate (DAS28-ESR) > 3.2, despite prior treatment with disease-modifying anti-rheumatic drugs (DMARDs) and/or biologics. All patients started with the TCZ standard regimen (8 mg/kg every 4 weeks iv) and concomitant DMARDs with or without prednisone, only with concomitant prednisone or TCZ as monotherapy. Adaptations in the TCZ regimen could be made, based on the expert opinion of the rheumatologist, in the case of: clinical inefficacy, adverse events, sustained low disease activity, or remission. Patients were eligible for inclusion in the final analyses if a serum sample (trough concentration) from at least one follow-up visit from week 12 onwards was available, taken after the TCZ standard regimen and in combination with the availability of corresponding measurements of DAS28 and/or CRP. The study was approved by the Medical Ethics Committee of Slotervaart Hospital and the Jan van Breemen Research Institute/Reade, Amsterdam, The Netherlands, and by the Medical Ethics Committee of La Paz Hospital, Madrid, Spain. All patients gave written informed consent in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Outcome measures

Disease activity was measured with the DAS28-ESR (19). In the Dutch cohort, DAS28, parameters of inflammation (CRP and ESR), and serum trough samples were collected at baseline and 4, 12, and 24 weeks thereafter. In the Spanish cohort, serum samples and parameters of inflammation were collected before every TCZ infusion whereas DAS28 and its separate components were measured at baseline and at 24 weeks. The duration of follow-up in this study was 24 weeks.

To investigate the relationship between serum TCZ trough concentration (described in the following as TCZ concentration) and clinical response at week 24, defined as an improvement compared to baseline in DAS28 (ΔDAS28) and swollen joint count in 28 joints (ΔSJC28). To obtain the concentration effect curve at week 24, the last observation carried forward (LOCF) method was used for patients in whom follow-up data from week 12 were available but not yet those from week 24. This seemed appropriate as the steady state of the TCZ standard regimen is seen, on average, from week 8 onwards (20). The relationship between TCZ concentration and serum CRP (described in the following as CRP) was investigated separately as CRP can be used as a surrogate marker for systemic IL-6R blockade (1, 6, 7).

TCZ concentration measurement

To measure TCZ concentrations an immunoassay was developed using rabbit anti-TCZ antibodies to capture TCZ, and rabbit anti-TCZ $F(ab')_2$ fragments for detection. Maxisorp enzyme-linked immunosorbent assay (ELISA) plates were coated overnight at room temperature with 0.125 μg/mL rabbit anti-TCZ in phosphate-buffered saline (PBS). The specific rabbit anti-idiotype antibodies were produced analogously as described for natalizumab (21). Plates were washed five times with PBS/0.02% Tween (PT), then washed and incubated for 1 h with patient serum that had been serially diluted in high-performance ELISA (HPE) buffer. After washing five times with PT, plates were incubated for 1 h with biotinylated anti-TCZ $F(ab')_2$ fragments (125 ng/mL in HPE buffer). After washing, streptavidin poly-horseradish peroxidase (poly-HRP; Sanquin, Amsterdam, The Netherlands) (1:10 000, in HPE buffer) was added for 1 h at 37 C. After washing, the ELISA was developed with 100 μg/mL tetramethylbenzidine in 0.11 M sodium acetate (pH 5.5) containing 0.003% (v/v) H_2O_2. The reaction was stopped with 2 M H_2SO_4. Absorption was measured at 450 nm relative to a titration curve of TCZ in each plate. The lower limit of quantification (LLOQ) in serum was 200 ng/mL; the overall precision and accuracy were 8% and 93%, respectively. Serum samples were collected at the trough concentration, that is just before the next infusion.

Anti-TCZ antibody measurement

Measurement of anti-TCZ antibodies was essentially carried out as described previously (22, 23). One

microlitre of serum diluted in buffer containing intravenous immunoglobulin (IVIg) $F(ab')_2$ to prevent anti-hinge reactivity (23) was incubated overnight with 1 mg Protein A Sepharose (GE Healthcare, Chalfont St Giles, UK) and 2.5 ng biotinylated $F(ab')_2$ TCZ in a final volume of 800 µL. Subsequently, samples were washed with 0.005% PT and about 1 ng ^{125}I-labelled streptavidin was added in a 800-µL final volume of PBS albumin Tween [PBS/0.01 M ethylenediaminetetraacetic acid (EDTA)/0.3% bovine serum albumin/0.004% Tween-20/0.05% NaN_3] and incubated overnight. Unbound label was removed by washing and Sepharose-bound radioactivity was measured. Antibody levels were compared to a standard serum sample of an immunized rabbit containing ADA and expressed in arbitrary units (AU). A lower limit of detection was based on mean +3 standard deviations (sd) measured in a panel of 50 sera from healthy donors and 15 sera containing anti-cyclic citrullinated peptide (anti-CCP) antibodies, antinuclear antibodis (ANA), and/or rheumatoid factor (RF).

Statistical analyses

For statistical analyses, SPSS version 21.0 and Graph Pad Prism 6.0 for Windows were used. The results are displayed as number and percentage or mean sd when normally distributed, or as median and interquartile range (IQR) when non-normally distributed. For differences in baseline characteristics between patients of the Spanish cohort vs. the Dutch, an independent-sample t-test, the Mann Whitney U test, or the χ^2 test was used, as appropriate. The threshold for significance was set at $p < 0.05$. Spearman s rank test was used to investigate the correlation between TCZ concentration and CRP. A linear regression analysis was used to investigate the relationship between TCZ concentration and ΔDAS28 at week 24. Potential confounders of this relationship were investigated using the baseline characteristics (Table 1), excluding the separate components of the DAS28, as the baseline DAS28 was already included. A variable was considered to be a confounder if it changed the regression coefficient by ≥ 10%. For this analysis, the LOCF method was used as described earlier. Sensitivity analyses showed no significant difference in baseline characteristics or outcome data (ΔDAS28 and TCZ concentration) at week 12 between patients with and without outcome data at week 24.

Results

The baseline characteristics of the 66 RA patients included in this study are shown in Table 1. Patients in the Spanish cohort had a significantly lower median ESR (mm/h) level of 23.5 (IQR 14.3 35.8) compared to 44 (IQR 22.5 63.0) for the Dutch patients ($p = 0.009$). The Dutch patients more often used ≥ 1 prior biological [34 (100%)] compared with the Spanish patients [19 (59,4%); $p < 0.001$].

Discontinuation and follow-up

A total of eight patients (12.1%) discontinued TCZ treatment between weeks 12 and 24 due to inefficacy

Table 1. Baseline demographics and clinical characteristics of the total patient population (n = 66).

Demographics	
Age (years)	56.0 12.9
Female	54 (81.8)
BMI (kg/m^2)	26.4 5.4
Spanish	32 (48.5)
Disease status	
Disease duration (years)	11.0 (5 17)
RF positive	47 (71.2)
Anti-CCP antibody positive	47 (71.2)
CRP (mg/L)	6.8 (2.1 31.9)
ESR (mm/h)	34 (16.5 47.5)
DAS28	5.4 1.4
Tender joint count	9.5 (4.0 14.5)
Swollen joint count	6.0 (2.8 10.3)
VAS GDA patient (0 100 mm)	60.0 25.8
DMARD therapy	
Prior biologicals	53 (80.3)
Methotrexate use at baseline	42 (63.6)
Methotrexate dose (mg/week)	15.5 7.3
Prednisone use at baseline	46 (69.7)
Prednisone dose (mg/day)	5.8 4.4
Other DMARD use (with or without methotrexate)	29 (43.9)

BMI, Body mass index; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, Disease Activity Score in 28 joints; VAS, visual analogue scale in mm (scale 0 100); GDA, general disease activity; DMARD, disease-modifying anti-rheumatic drug.
Values given as number (percentage), mean standard deviation, or median (interquartile range).

Table 2. Median serum tocilizumab (TCZ) trough concentrations during the 24 weeks of follow-up.

	Week 4	Week 12	Week 24
Number (%) of patients on TCZ	66 (100)	66 (100)	55 (83.3)
Number (%) of available samples	49 (74.2)*	62 (93.9)*	53 (96.4)
Median TCZ (mg/L)	3.4	9.1	10.6
Minimum TCZ (mg/L)	0	0	0
Maximum TCZ (mg/L)	18.2	35.5	35.4
Number (%) of patients with TCZ < 1 mg/L	15 (30.6)	15 (24.2)	9 (17.0)

* Percentage is based on the number of patients on TCZ.
Percentage is based on the number of available samples at each time point.

(n = 5) or adverse events (n = 3). For three patients, clinical data and serum from week 12 were available but they had not yet had 24 weeks of follow-up at the time of data extraction.

Serum TCZ concentration and anti-TCZ antibodies

The available samples and median TCZ concentrations at each time point are shown in Table 2, with the lowest and highest concentrations and the number of patients with TCZ concentrations below 1 mg/L.

In total, nine patients had TCZ concentrations below 1 mg/L at ≥ 2 subsequent visits, of which there were three patients at every time point. This means that some patients had TCZ concentrations below 1 mg/L repeatedly, despite receiving the TCZ standard regimen. However, this could not be established in all patients because of some missing samples, and therefore this number might be an underestimation. Although, TCZ concentrations below 1 mg/L were found (repeatedly) in several patients, an anti-TCZ antibody signal was seen in only two patients with the assay used. In one of these patients, a weak anti-TCZ antibody signal was detected consistently, including in the pretreatment samples, independently of TCZ concentration. This patient was therefore not considered anti-TCZ antibody positive. A sample from the other patient was found to be positive at week 4, which was confirmed by subsequent inhibition experiments using unlabelled TCZ $F(ab')_2$. This patient had TCZ concentrations below 1 mg/L at week 4; these had increased to ≥ 1 mg/L at week 24 with subsequent normalization of serum CRP levels.

Serum TCZ concentration, inflammation parameters, and disease activity

At baseline, 26 patients (39.4%) had a CRP level above 10 mg/L with a median of 37.7 mg/L (IQR 21.9–49.7). To investigate the relationship between TCZ concentration and CRP levels, all samples were stratified from low to high according to TCZ concentration with correlating CRP levels, as shown in Figure 1. This figure includes all samples from week 4 onwards, thus one dot represents one sample and not one patient. Based on this figure, a TCZ concentration above 1 mg/L is sufficient to normalize serum CRP levels (≤ 10 mg/L). Spearman s rank correlation coefficient was calculated (based on the sample included in Figure 1) and showed a significant but moderately strong negative correlation coefficient of −0.460 ($p < 0.0001$).

To provide an insight into the course of CRP normalization over time with the corresponding TCZ concentration, Figure 1 was also divided at each time point (i.e. weeks 4, 12, and 24), see Supplementary Material. This supplementary figure shows that only one patient had an elevated CRP level at the time point of drop-out (week 12) and in almost all other patients CRP normalized over time. None of the patients had increased CRP levels during follow-up in combination with TCZ > 1 mg/L at any time point.

A similar course of improvement was seen for ESR; nevertheless, the cut-off of TCZ of 1 mg/L was less marked compared to CRP. With TCZ concentrations above 6 mg/L, no increased ESR levels (> 20 mm/h) were found (data not shown).

Concentration effect curve of TCZ at week 24

In Figure 2, the relationship between TCZ concentration and ΔDAS28 (Figure 2A) and ΔSJC28 (Figure 2B) for

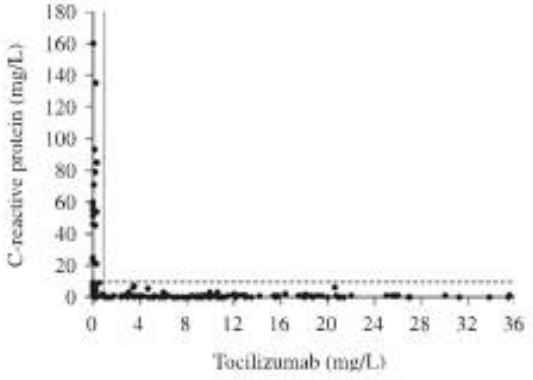

Figure 1. The relationship between Tocilizumab (TCZ) concentration (mg/L) and C-reactive protein (CRP) levels (mg/L). All samples from week 4 onwards were stratified from low to high according to the TCZ concentration with correlating CRP levels; thus one dot represents one sample and not one patient. Based on this figure, a TCZ concentration above 1 mg/L (marked with a vertical broken line) is sufficient to normalize serum CRP levels (≤ 10 mg/L) (marked with a horizontal broken line). Spearman s rank correlation showed a significant but moderately strong negative correlation coefficient of −0.460 ($p < 0.0001$).

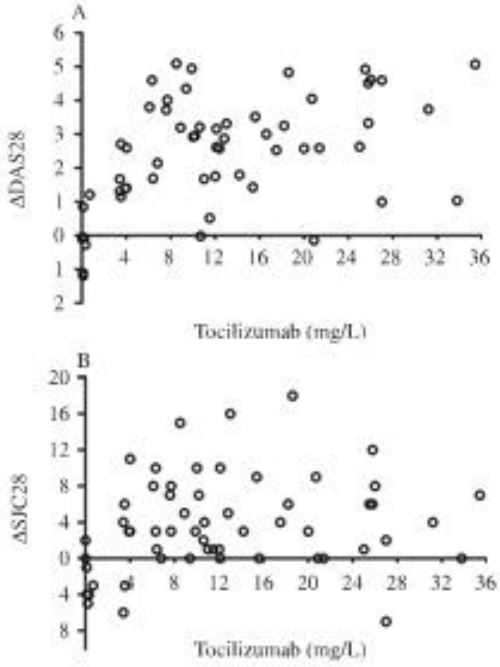

Figure 2. Concentration effect curves, assessed at 24 weeks of treatment, of tocilizumab (TCZ) with corresponding improvement in (A) the Disease Activity Score in 28 joints (ΔDAS28) and (B) the swollen joint count in 28 joints (ΔSJC28) compared to baseline.

the individual patients at week 24 is presented. Twelve patients (18%) did not achieve a ΔDAS28 of ≥ 1.2, which is considered to be a clinically significant change. Eight of these 12 patients had a TCZ concentration < 1 mg/L. In addition, three of these eight patients had a CRP level above 10 mg/L. Linear regression analysis showed a regression coefficient of 0.080 with a 95% confidence interval (CI) of 0.039 0.113 ($p < 0.001$) for the association between TCZ concentration and ΔDAS28 at week 24 of treatment. No confounders were identified.

Eight patients had more swollen joints at week 24 of treatment compared to baseline, of whom five had a TCZ concentration below 1 mg/L. Two of these five patients had increased CRP levels, two other patients had increased CRP levels at previous time points, and one had normal CRP levels. In general, the SJC had improved or stabilized in the majority of patients with TCZ concentrations of approximately 4 mg/L.

Discussion

The aim of this study was to investigate, in a prospective observational cohort, the variation in serum TCZ trough concentrations and the relationship with clinical measurements in RA during 24 weeks of follow-up. This study shows that serum TCZ trough concentrations vary widely between patients on the TCZ standard regimen, as was seen previously in anti-TNF treatment. Moreover, the majority of patients obtained TCZ concentrations that were sufficient to normalize serum CRP. A statically significant ΔDAS28 improvement with increasing TCZ concentrations was seen, but for clinical implications this change was small.

For anti-TNF treatment it has been shown that immunogenicity can have a profound effect on the PK (16). However, the PK of TCZ appears to be different because anti-TCZ antibodies were detected only in one patient, although TCZ concentrations below 1 mg/L were found (repeatedly) in several patients. Moreover, these low concentrations were especially evident during the early treatment phase, which is not in accordance with reported PK variations due to immunogenicity in anti-TNF treatment. The influence of immunogenicity might have been underestimated in this study as the assay used to detect anti-TCZ antibodies is drug sensitive, meaning that only ADA exceeding TCZ concentration will be detected (24, 25). Previously reported data from RCTs suggest that immunogenicity is not a major factor in the PK of TCZ (10, 11, 13), but these trials included a different patient population from that encountered in daily clinical practice. Moreover, comparing ADA results is difficult because ADA production and detection can be influenced by several factors, such as time point of sampling, assay format, concomitant immunomodulation therapy (e.g. methotrexate), and dosing (26). Thus, it remains open to question whether TCZ has a less immunogenic structure, or detectability is more complex due to drug interference, or immunological tolerance is induced by high dosing (27, 28). Therefore, it would be of interest to investigate the immunogenicity of TCZ with a drug tolerant assay similar to that performed previously for adalimumab (29, 30).

Another explanation for the variation in TCZ concentration between patients is target binding. In patients with more target, (i.e. IL-6R), clearance of TCZ is increased, and thus lower serum TCZ trough concentrations will be detected by the assay. Moreover, a TCZ concentration above 1 mg/L was sufficient to normalize CRP levels, and Spearman s rank test showed a statistically significant moderately strong negative correlation between TCZ and CRP. CRP is mainly produced by hepatocytes through IL-6 activation and can therefore be used as a surrogate marker for systemic IL-6R binding (1, 6, 7). Target binding that influence the PK of TCZ has been suggested previously in several studies. Inhibition assays have shown that the binding between IL-6 and sIL-6R was suppressed, in a dose-dependent manner, by adding TCZ at concentrations between 0.002 and 4 mg/L (31). Nishimoto et al show that TCZ concentrations above 1 mg/L resulted in more than 95% binding of sIL-6R in an sIL-6R/TCZ immune complex with subsequent inhibition of CRP production (12). Another clinical trial showed that these TCZ concentrations were obtained in the majority of patients from week 8 onwards (11). In addition, the TCZ concentration

at which 50% of its maximal effect was observed was lower in patients with high IL-6 levels at baseline, which may be the result of IL-6 overproduction or lower expression of mIL-6R, and thus slower clearance (14). Different amounts of target, in the normal or inflammatory state, might be explained by sIL-6R polymorphisms (32, 33); however, to our knowledge, this was not studied in combination with serum TCZ concentrations.

Although CRP can be used as a surrogate marker for systemic IL-6R binding, clinical response is more complex. Clinical response is multifactorial and clinical outcome measurements frequently used in RA [such as DAS28, the Clinical Disease Activity Index (CDAI) (34), or the Simplified Disease Activity Index (SDAI) (35)] are composite measurements that reflect total disease activity but do not discriminate between the role of a particular cytokine and other factors (e.g. other cytokines, established bone damage, psychological and social factors). Linear regression analysis showed a statistically significant ΔDAS28 improvement with increasing TCZ concentrations; however, for clinical purposes this change was small. Moreover, normalized CRP did not result in a good clinical outcome, measured with DAS28 or SJC28, in all patients. In addition, the predictive value of IL-6, sIL-6R, or CRP on clinical outcome is contradictory (12, 13, 17, 36, 37). Nevertheless, TCZ, like all biologics given in RA, is a molecular targeting therapy and thus the highest obtainable result is complete target blockade; however, this does not necessarily translate to an appropriate clinical response in all patients. The assay used for TCZ concentrations measures the surplus of unbound TCZ, and a detectable serum trough concentration means that all systemic, and potentially all local (38, 39), IL-6R is blocked. Therefore, TCZ concentration measurements can be used as a surrogate marker for systemic target blockade. Considering the number of non-responders to biological treatment and the high costs associated with these therapies, a dose based on target levels seems more rational. To apply therapeutic drug monitoring (TDM) of biologicals in daily clinical practice for treatment optimization, an optimal therapeutic concentration range for an effective target blockade must be identified (40, 41). Because of the direct relationship between IL-6 and CRP, TCZ is a suitable biological agent for investigating the optimal therapeutic concentration range for complete target blockade vs. clinical response. To investigate the additional value of TDM, a prospective TCZ dose taper trial is necessary, including clinical measurements, TCZ concentrations and other potential markers, for target blockade (IL-6, sIL-6R, CRP, and calprotectin) (12, 13, 17, 35, 36, 42–44), as well as biomarkers for progression of bone damage because IL-6 plays an important role in bone metabolism (3, 45).

Some limitations of the current study need to be addressed. Two cohorts were combined to increase patient numbers, resulting in slight differences in measurements per time point. Differences in patient characteristics were limited and both cohorts consisted of mainly Caucasians. However, possible bias due to non-compliance, change in TCZ dose, or interval or wrong timing of sampling was excluded since TCZ was given intravenously.

In conclusion, the TCZ standard regimen results in a wide variety of serum TCZ trough concentrations between patients, and target binding seems to provide a better explanation for this variation than immunogenicity. Moreover, the majority of patients obtained TCZ concentrations above 1 mg/L, which is sufficient to normalize serum CRP. Therefore, the TCZ standard regimen is an overtreatment with regard to systemic IL-6R blockade in the majority of RA patients; however, clinical response is multifactorial and might require more than just sufficient blockade of a single cytokine pathway.

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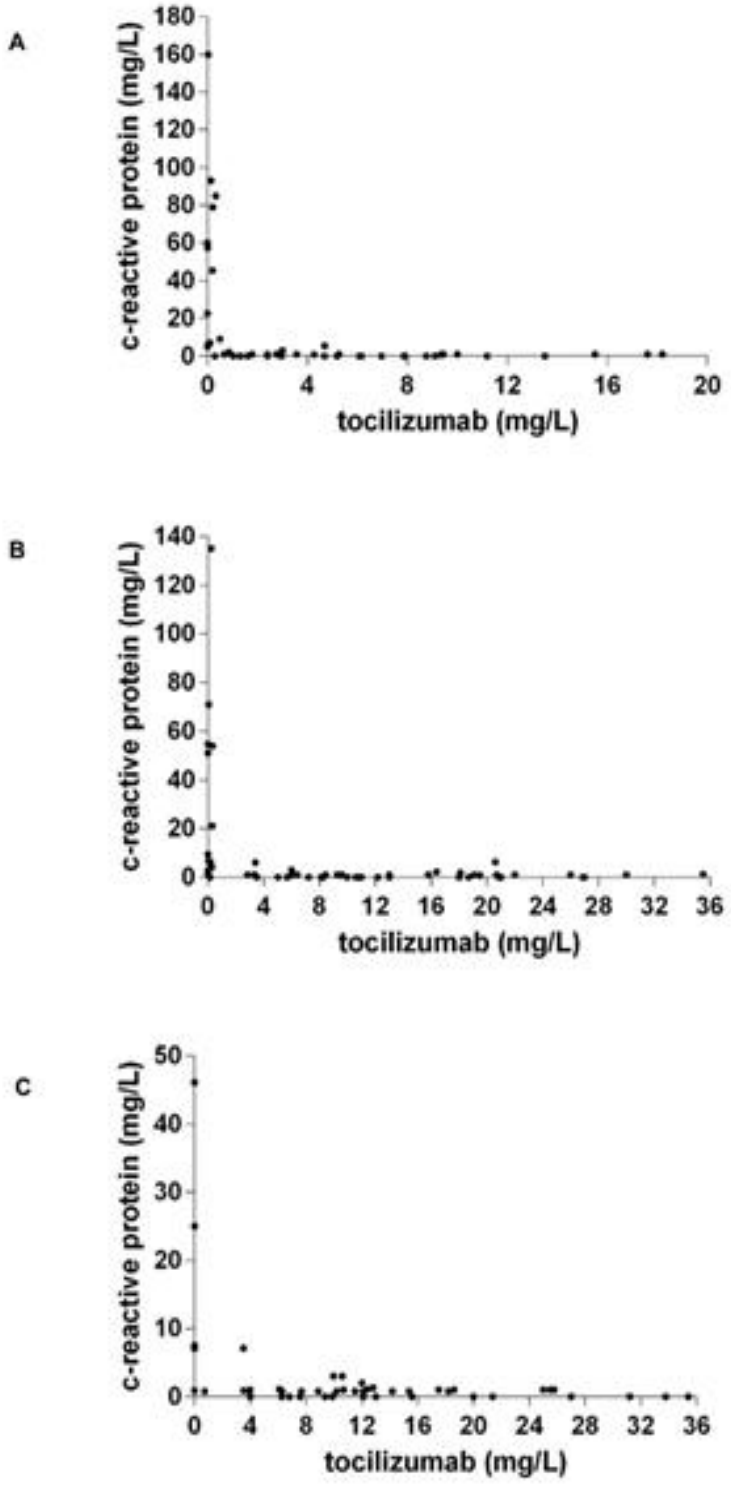
Supplementary figure S1



For this figure the same procedure was followed as for figure 1 (main text), but, figure 2 represents the relationship between tocilizumab (TCZ) concentrations (mg/L) with correlating C-reactive protein (CRP) levels (mg/L) separated per time point (week 4 (A), week 12 (B) and week 24 (C)). Thus, one dot represents one patient.

ARTICLE 6

TITLE: ***"Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical Practice"***

JOURNAL: Ann Rheum Dis 2014;73(12):2217–9(102)

AUTHORS: Kneepkens, Eva L.; **Plasencia, Chamaida**; Krieckaert, Charlotte L. M.; Pascual-Salcedo, Dora; van der Kleij, Desiree; Nurmohamed, Michael T.; Teresa Lopez-Casla, M.; Wieringa, Roeland; Rispens, Theo; Wolbink, Gertjan.

PATIENTS AND METHODS:

This prospective observational cohort consisted of 37 consecutive adult patients with RA, according to the American College of Rheumatology 1987 revised criteria, in whom Goli 50 mg subcutaneously once monthly was initiated, and who were recruited from two departments (Spain and The Netherlands). The study was approved by both Medical Ethics Committees. Clinical response was defined as DAS28-ESR <3.2. Patients were eligible for inclusion when clinical data and sera of baseline with ≥ one follow-up visit were available. Clinical measurements and trough-level sera were collected at baseline and 4, 16, 28 and 52 weeks (The Netherlands), or half yearly (Spain), thereafter. Goli levels were measured analogously to adalimumab using TNF for capture and rabbit anti-Goli for detection (lower limit of quantification (LLOQ) 5 nag/ mL; accuracy 103%, precision 12%). ADA were measured, using an ADA radio-immune assay, described previously. Cut-off (mean+3 SD) was based on a serum panel of 80 healthy donors and 15 sera containing ACPA, ANA, and/or RF. For statistical analysis SPSS V.17.0 and Graph Pad Prism 5 for windows were used. Threshold for significance was set at $p<0.05$. To analyze the association between Goli levels and response at 1 year, last observation carried forward was used for patients who discontinued Goli treatment prematurely.

Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice

Tumour necrosis factor inhibitors (TNFi) are effective in the majority of patients with rheumatoid arthritis (RA),[1] however, an important reason for non-response is low drug level due to immunogenicity.[2] To our knowledge, no data collected during a prospective observational study is currently available regarding the relationship between golimumab level, immunogenicity and response in RA.

This prospective observational cohort consisted of 37 consecutive adult patients with RA, according to the American College of Rheumatology 1987 revised criteria,[3] in whom golimumab 50 mg subcutaneously once monthly was initiated according to the judgment of the rheumatologist, and who were recruited from two departments (Spain and The Netherlands). The study was approved by both Medical Ethics Committees. Clinical response was defined as Disease Activity Score using 28 joint count (DAS28) <3.2, calculated with erythrocyte sedimentation rate (ESR) (mm/h). Patients were eligible for inclusion when clinical data and sera of baseline with ≥ one follow-up visit were available.

Clinical measurements and trough-level sera were collected at baseline and 4, 16, 28 and 52 weeks (The Netherlands), or half yearly (Spain), thereafter. Golimumab levels were measured analogously to adalimumab[4] using TNF for capture and rabbit antigolimumab for detection (lower limit of quantification (LLOQ) 5 ng/mL; accuracy 103%, precision 12%). Antidrug antibodies (ADA) were measured, using an ADA radio-immune assay, described previously.[4 5] Cut-off (mean+3 SD) was based on a serum panel of 80 healthy donors and 15 sera containing anticyclic citrullinated peptide (anti-CCP), ANA, and/or rheumatoid factor. All baseline samples were ADA against golimumab negative.

For statistical analysis SPSS V17.0 and Graph Pad Prism 5 for windows were used. Threshold for significance was set at $p<0.05$. To analyse the association between golimumab level and response at 1 year, last observation carried forward was used for patients who discontinued golimumab treatment prematurely.

For baseline characteristics, see table 1.

At week 52, 15 patients (40.5%) were responder and 22 (59.5%) non-responder. Nineteen patients (51.4%) discontinued golimumab treatment prematurely due to inefficacy (11), side

Table 1 Baseline characteristics

	Total patient population n=37	DAS28 <3.2 n=15	DAS28 ≥3.2 n=22
Demographics			
Age, years	51.7 13.9	50.4 15.5	52.6 12.9
Female, number (%)	31 (83.8)	10 (66.7)	21 (95.5)*
BMI (median)	25.5 (22.7 27.3)	23.5 (20.7 26.8)	26 (23.2 29)
Disease status			
Disease duration, years (mean)	12.3 8.7	11.6 10.3	12.8 7.7
Rheumatoid factor positive, number (%)	25 (67.6)	9 (60)	16 (72.7)
Anti-CCP positive, number (%)	22 (59.5)	8 (53.3)	14 (63.6)
Erosive disease, number (%)	18 (48.6)	7 (46.7)	11 (50)
CRP mg/L (median)	6.4 (2 18)	2 (1 19)	9 (2 18)
ESR mm/h (median)	29 (7.5 42)	7 (4 38)	34 (21.8 48)**
DAS28 (mean)	4.4 1.3	3.1 1	5.1 1.1***
DMARD therapy			
Prior biologicals, number (%)	24 (64.9)	8 (53.3)	16 (72.7)
Methotrexate use, number (%)	24 (64.9)	11 (73.3)	13 (59.1)
Methotrexate dose (mg/wk)	20 (10.6 25)	25 (15 25)	12.5 (8.8 25)
Prednisone use, number (%)	16 (43.2)	6 (40)	10 (45.5)
Prednisone dose (mg/day)	5 (5 10)	6.3 (4.3 10)	5 (5 10)

Normally distributed continuous variables are represented by mean values SD and non-normally distributed continuous variables are represented by median values (IQR); dichotomous variables are represented by numbers (percentages of total).
For differences between groups at baseline, the independent sample t test was used for normally distributed continuous variables, Mann Whitney U test was used for non-normally distributed continuous variables, and the χ^2 test was used for dichotomous variables.
*p=0.02; **p=0.02; ***p<0.001.
BMI, Body Mass Index; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, Disease Activity Score using 28-joint count; DMARD, disease modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate.

Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice

Tumour necrosis factor inhibitors (TNFi) are effective in the majority of patients with rheumatoid arthritis (RA),[1] however, an important reason for non-response is low drug level due to immunogenicity.[2] To our knowledge, no data collected during a prospective observational study is currently available regarding the relationship between golimumab level, immunogenicity and response in RA.

This prospective observational cohort consisted of 37 consecutive adult patients with RA, according to the American College of Rheumatology 1987 revised criteria,[3] in whom golimumab 50 mg subcutaneously once monthly was initiated according to the judgment of the rheumatologist, and who were recruited from two departments (Spain and The Netherlands). The study was approved by both Medical Ethics Committees. Clinical response was defined as Disease Activity Score using 28 joint count (DAS28) <3.2, calculated with erythrocyte sedimentation rate (ESR) (mm/h). Patients were eligible for inclusion when clinical data and sera of baseline with ≥ one follow-up visit were available.

Clinical measurements and trough-level sera were collected at baseline and 4, 16, 28 and 52 weeks (The Netherlands), or half yearly (Spain), thereafter. Golimumab levels were measured analogously to adalimumab[4] using TNF for capture and rabbit antigolimumab for detection (lower limit of quantification (LLOQ) 5 ng/mL; accuracy 103%, precision 12%). Antidrug antibodies (ADA) were measured, using an ADA radio-immune assay, described previously.[4 5] Cut-off (mean+3 SD) was based on a serum panel of 80 healthy donors and 15 sera containing anticyclic citrullinated peptide (anti-CCP), ANA, and/or rheumatoid factor. All baseline samples were ADA against golimumab negative.

For statistical analysis SPSS V.17.0 and Graph Pad Prism 5 for windows were used. Threshold for significance was set at $p<0.05$. To analyse the association between golimumab level and response at 1 year, last observation carried forward was used for patients who discontinued golimumab treatment prematurely.

For baseline characteristics, see table 1.

At week 52, 15 patients (40.5%) were responder and 22 (59.5%) non-responder. Nineteen patients (51.4%) discontinued golimumab treatment prematurely due to inefficacy (11), side

Table 1 Baseline characteristics

	Total patient population n=37	DAS28 <3.2 n=15	DAS28 ≥3.2 n=22
Demographics			
Age, years	51.7 13.9	50.4 15.5	52.6 12.9
Female, number (%)	31 (83.8)	10 (66.7)	21 (95.5)*
BMI (median)	25.5 (22.7 27.3)	23.5 (20.7 26.8)	26 (23.2 29)
Disease status			
Disease duration, years (mean)	12.3 8.7	11.6 10.3	12.8 7.7
Rheumatoid factor positive, number (%)	25 (67.6)	9 (60)	16 (72.7)
Anti-CCP positive, number (%)	22 (59.5)	8 (53.3)	14 (63.6)
Erosive disease, number (%)	18 (48.6)	7 (46.7)	11 (50)
CRP mg/L (median)	6.4 (2 18)	2 (1 19)	9 (2 18)
ESR mm/h (median)	29 (7.5 42)	7 (4 38)	34 (21.8 48)**
DAS28 (mean)	4.4 1.3	3.1 1	5.1 1.1***
DMARD therapy			
Prior biologicals, number (%)	24 (64.9)	8 (53.3)	16 (72.7)
Methotrexate use, number (%)	24 (64.9)	11 (73.3)	13 (59.1)
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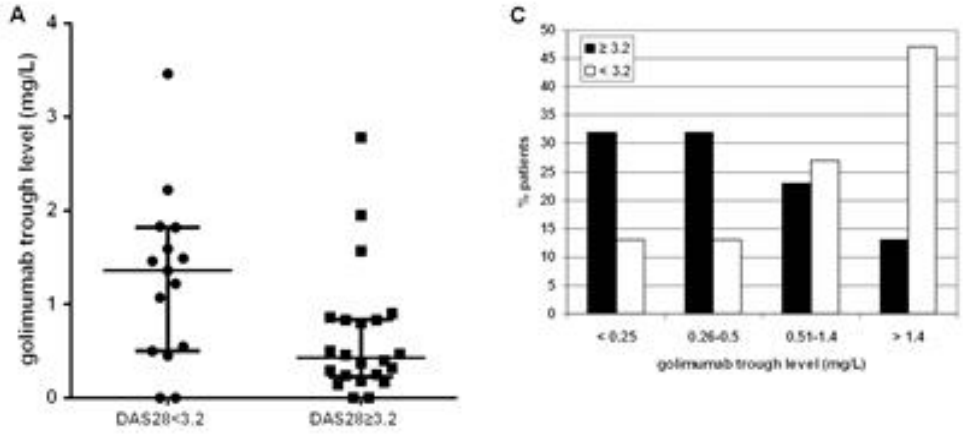
Prednisone use, number (%)	16 (43.2)	6 (40)	10 (45.5)
Prednisone dose (mg/day)	5 (5 10)	6.3 (4.3 10)	5 (5 10)

Normally distributed continuous variables are represented by mean values SD and non-normally distributed continuous variables are represented by median values (IQR); dichotomous variables are represented by numbers (percentages of total).
For differences between groups at baseline, the independent sample t test was used for normally distributed continuous variables, Mann Whitney U test was used for non-normally distributed continuous variables, and the χ^2 test was used for dichotomous variables.
*p=0.02; **p=0.02; ***p<0.001.
BMI, Body Mass Index; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS28, Disease Activity Score using 28-joint count; DMARD, disease modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate.



B

	RC	95%-CI	p-value	RC*	95%-CI*	p-value*
DAS28	-0.49	-0.93 to -0.05	0.03	-0.42	-0.88 to 0.04	0.07
ESR**	-9.03	-14.2 to -3.9	0.001	-7.5	-13.7 to -1.3	0.02
CRP**	-0.46	-0.60 to -0.28	p<0.001	-0.44	-0.59 to -0.24	<0.001

RC= regression coefficient; 95%-CI= 95% coincidence interval; DAS28= disease activity score using 28 joint count; ESR=erythrocyte sedimentation rate; CRP= C-reactive protein.
*values after corrected for the baseline value.
** ESR and CRP were not normally distributed, however, for the association between golimumab level and ESR GEE was used without transformation of the data into logarithms for normality, since standardized residuals were normally distributed.

Figure 1 (A) Median golimumab trough level (mg/L) (with IQR) was higher in patients with a DAS28 <3.2 versus ≥3.2 at week 52 of treatment (p=0.023). (B) The association between golimumab trough level and disease activity over time during one year of follow-up, analysed with a generalised estimating equation. (C) Percentage of patients with DAS28 <3.2 and ≥3.2 stratified according to the golimumab level at 52 weeks of treatment. Each group contains 9 patients (25% of all patients) and the last quartile 10.

effects (7) or other reasons (1); with a median drug survival of 16 weeks. Median golimumab level (mg/L) at week 52 was 0.55 (0.27 1.48), and was significantly higher, analysed with a χ^2 test, in responders, 1.36 (0.5 1.82), compared with non-responders, 0.43 (0.23 0.84) (p=0.023) (figure 1A). Generalised estimating equation analysis demonstrated, after adjustment for baseline values, a statistically significant inverse association between golimumab level and C-reactive protein (CRP) (mg/L)/ESR. After correction, a trend remained visible for DAS28 (figure 1B).

All patients were stratified according to the golimumab level at week 52 and divided into quartiles (figure 1C). The lowest quartile (golimumab <0.25 mg/L) comprised 32% of all non-responders, while, the highest (golimumab >1.4 mg/L) comprised 47% of all responders.

During 52 weeks, 3 patients were ADA positive (ADA >12 AU/mL one ≥1 occasion in combination with golimumab levels <0.1 mg/L).[4] All three patients discontinued golimumab prematurely due to inefficacy. One patient used concomitant methotrexate. However, the assay used to detect ADA can be influenced by drug interference, resulting in an underestimation of ADA.[6] The percentages of patients with ADA against golimumab found in prior studies varied between 2.1% to 13%.[7 10] However, head-to-head comparison of ADA percentages is complicated, since several factors can influence immunogenicity[2] and clinical non-response is multifactorial.

In conclusion, responders had a significantly higher golimumab trough level at 1 year of treatment. ESR and CRP were statistically significantly inversely associated with golimumab level over time. Three patients had high ADA titres resulting in undetectable golimumab levels, and thus in a poor clinical outcome. These results can be used to further optimise golimumab treatment in RA.

There are some limitations to this study: limited patient number, the majority of patients used prior TNFi, and golimumab discontinuation rate was relatively high.

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Contributors Study concept and design: ELK, CP, CLMK, DP-S, DvdK, MTN, MTL-C, RW, TR, GW. Acquisition of data: ELK, CP, DP-S. Analysis and interpretation of the data: ELK, CP, GW. Clinical revision and drafting of the manuscript for important intellectual content: ELK, CP, CLMK, DP-S, Dv dK, MTN, MTL-C, RW, TR, GW. Obtained funding: none. Study supervision: MTN, GW. Final approval: ELK, CP, CLMK, DP-S, DvdK, MTN, MTL-C, RW, TR, GW.

Competing interests MTN reports having received consultancy fees from Abbott, Roche, Pfizer, MSD, UCB, SOBI and BMS, payment for lectures from Abbott, Roche and Pfizer. GW reports having received a research grant from Pfizer (Wyeth) (paid to the institution) and payments for lectures from Pfizer, UCB, Abbvie and Amgen. CLMK reports having received payment for lectures from AbbVie and Pfizer. TR reports having received payment for lectures from AbbVie and Pfizer. CP reports

having received a research grant from Pfizer. DP-S reports having received payments for lectures from Pfizer and a research grant from Pfizer. ELK reports having received payments for lectures from Pfizer. DvdK, MTL-C and RW have no disclosures

Patient consent Obtained.

Ethics approval The study was approved by the Medical Ethics Committee of the Slotervaart Hospital and Jan van Breemen Research Institute | Reade, Amsterdam (The Netherlands) and La Paz Hospital, Madrid (Spain).

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Golimumab trough levels, antidrug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice

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Chapter 2: Therapeutic strategies based on monitoring of drug and ADA levels

ARTICLE 7: ***"The immunogenicity to the first anti-TNF therapy determines the outcome of switching to a second anti-TNF therapy in Spondyloarthritis patients"***

ARTICLE 8: ***"Effect of infliximab dose increase in rheumatoid arthritis at different trough concentrations: a cohort study in clinical practice conditions"***

ARTICLE 7

TITLE:"*The immunogenicity to the first anti-TNF therapy determines the outcome of switching to a second anti-TNF therapy in spondyloarthritis patients*"

JOURNAL: Arthritis Res Ther 2013;15(4):R79(118)

AUTHORS: Plasencia, Chamaida; Pascual-Salcedo, Dora; Garcia-Carazo, Sara; Lojo, Leticia; Nuno, Laura; Villalba, Alejandro; Peiteado, Diana; Arribas, Florencia; Diez, Jesus; Teresa Lopez-Casla, Maria; Martin-Mola, Emilio; Balsa, Alejandro.

PATIENTS AND METHODS:

Patients and sera

A total of 42 SpA patients from SpA-Paz cohort without previous biological treatment were included. This was an ambispective observational study that was approved by the La Paz Hospital Ethics Committee, and all patients provided informed written consent. All of the AS patients fulfilled the New York revised criteria for AS [23]. The psoriatic arthritis patients fulfilled the GRAPPA group criteria.

All patients received anti-TNF drugs as a first biological treatment Ifx, Ada and Etn and later switched to a second anti- TNF drug (Ifx, Ada, Etn and Goli). All biologics were administered at standard therapy regimen.

Disease activity was measured by ASDAS and was assessed at baseline and every 6 months. Clinically important improvement was defined as change in ASDAS ≥1.1. Data related to the clinical activity in the retrospective period were obtained from our database of patients on biological therapy.

Blood samples were collected a maximum of 24 hours before biological drug administration for subcutaneous anti-TNF or just before intravenous infusion for Ifx.

Measurement of drug and anti-drug antibody concentrations

The serum drug concentrations (Ifx, Ada and Etn) were determined by sandwich ELISA, as described previously. Serum drug levels were considered positive for Ifx if >10 nag/ml, for Ada if >5 nag/ml and for Etn if >30 nag/ml. Serum ADA levels (antibodies to Ifx, antibodies to Ada and antibodies to Etn) were detected using a two-site (bridging) ELISA, as previously described. The cutoff value for the presence of antibodies to Ifx was established at 50 AU/ml, for antibodies to Ada at 10 AU/ml and for antibodies to Etn at 50 AU/ml.

Statistical analysis

The statistical analyses were performed using the Statistical Package for the Social Sciences, version 11.0 (SPSS, Chicago, IL, USA). Descriptive statistics included the mean and standard deviation or the median and interquartile range. Differences in baseline characteristics were assessed using Pearson's chi-square test and Fisher's exact test for ordinal variables and using the Mann- Whitney U test for continuous variables. The continuous data were compared between groups using the Mann-Whitney U test. Statistical significance was calculated using the log-rank test, and $p < 0.05$ was considered significant.

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RESEARCH ARTICLE Open Access

The immunogenicity to the first anti-TNF therapy determines the outcome of switching to a second anti-TNF therapy in spondyloarthritis patients

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Abstract

Introduction: Anti-TNF drugs have proven to be effective against spondyloarthritis (SpA), although 30% of patients fail to respond or experience adverse events leading to treatment discontinuation. In rheumatoid arthritis, the presence of anti-drug antibodies (ADA) against the first TNF inhibitor influences the outcome after switching. Our aim was to assess whether the response to a second anti-TNF drug is related to the previous development of ADA to the first anti-TNF drug SpA patients.

Methods: Forty-two SpA patients began a second anti-TNF drug after failing to respond to the first anti-TNF therapy. Clinical activity was assessed by the Ankylosing Spondylitis Disease Activity Score (ASDAS) at baseline (at the beginning of the first and second anti-TNF therapy) and at 6 months after switching. The drug and ADA levels were measured by ELISA before each administration.

Results: All patients were treated with anti-TNF drugs and mainly due to inefficacy were switched to a second anti-TNF drug. Eleven of 42 (26.2%) developed ADA during the first biologic treatment. At baseline, no differences in ASDAS were found in patients with or without ADA to the first anti-TNF drug (3.52 1.03 without ADA vs. 3.14 0.95 with ADA, $p = 0.399$) and to the second anti-TNF drug (3.36 0.94 without ADA vs. 3.09 0.91 with ADA, $p = 0.466$). At 6 months after switching, patients with previous ADA had lower disease activity (1.62 0.93 with ADA vs. 2.79 1.01 without ADA, $p = 0.002$) and most patients without ADA had high disease activity state by the ASDAS (25 out of 31 (80.6%) without ADA vs. 3 out of 11 (27.3%) with ADA, $p = 0.002$).

Conclusions: In SpA the failure to respond to the first anti-TNF drug due to the presence of ADA predicts a better clinical response to a second anti-TNF drug.

Introduction

Spondyloarthritis (SpA) describes a group of diseases including ankylosing spondylitis (AS), psoriatic SpA, SpA related to inflammatory bowel disease (IBD), reactive arthritis, a subgroup of juvenile idiopathic arthritis and nonradiographic axial spondyloarthritis [1]. Several studies have demonstrated the efficacy of biological agents, such as anti-TNFα drugs, for treating SpA patients [2-9].

The available anti-TNF drugs differ in chemical structure, half-life, route of application and capacity to induce immunogenicity, and they also have somewhat different mechanisms of action [10,11]. Although the efficacy of anti-TNF drugs against SpA has been shown in large, randomised clinical trials [6,12-16], it is known that some patients fail to respond to treatment or experience adverse events necessitating treatment

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Results

Patient characteristics

A total of 42 SpA patients were enrolled in this study with a mean standard deviation age of 49.6 10.4 years at the time of inclusion, and 23 (54.8%) were men. The baseline demographic and clinical characteristics of the global patient population, categorised according to future ADA development against the first anti-TNF therapy, are shown in Table 1. No differences in patient characteristics were present at baseline between those who later developed ADA and those who did not (Table 1).

Immunogenicity in relation to biological therapy

All 42 patients were treated with an anti-TNF drug as the first biologic therapy (20 with Ifx, 5 with Ada and 17 with Etn) and were switched to a second anti-TNF treatment (9 Ifx, 19 Ada, 8 Etn and 6 Gol) due to inefficacy (39 out of 42 patients, 92.8%; 11 of them ADA-positive) and/or adverse events (8 out of 42 patients, 19%). Of the eight patients who withdrew due to adverse events, three had been treated with Ifx (all of them with ADA and clinical inefficacy, having infusion-related reactions) and five with Etn (two out of five patients also had clinical inefficacy, all of them having local injection reaction and/or pruritus).

ADA were detected in 11 (26.2%) patients (7/27 (25.9%) AS, 3/10 (30%) undifferentiated SpA and 1/2 (50%) SpA related to IBD) during treatment with the first anti-TNF drug and were more frequent in patients treated with Ifx (9 (81.8%) with Ifx, 2 (18.2%) with Ada, 0 (0%) with Etn, $p = 0.006$). ADA appeared mainly within the first year of anti-TNF therapy (mean standard deviation: 12.89 5.92 months), except for five patients in whom ADA were detected at 18 months (2 patients), 20 months (2 patients) and 28 months (1 patient). At 6 months after switching to the second anti-TNF drug, ADA were detected in only two patients treated with Ifx, who had not previously developed antibodies against the first anti-TNF drug. The drug and ADA concentrations were not evaluated for Gol.

Table 1 Demographic characteristics of 42 spondyloarthritis patients

Characteristic	Total (42 patients)	Without ADA (31 patients)	With ADA (11 patients)	p value
Sex, male	23 (54.8%)	16 (51.6%)	7 (63.6%)	0.726
Age	49.60 10.46	51.26 10.08	44.91 10.53	0.084
HLA-B27-positive[a]	23/36 (64%)	17/27 (63%)	6/9 (66.7%)	0.841
Disease duration (years)	12.24 8.23	11.61 8.04	14 8.92	0.416
Baseline ASDAS	3.42 1.01	3.52 1.03	3.14 0.95	0.399
Concomitant treatment				
Methotrexate	9 (21.5%)	8 (25.8%)	1 (9.1%)	0.498
Other DMARDs	10 (23.8%)	5 (16.1%)	5 (45.4%)	0.115
Methotrexate + other	1 (4.7%)	2 (6.4%)	0 (0%)	0.599
DMARDs				
Monotherapy	21 (50%)	16 (51.7%)	5 (45.5%)	0.126
Corticosteroid therapy	15 (35.7%)	9 (29%)	6 (50%)	

Data presented as n (%) or mean standard deviation. ADA, anti-drug antibodies; ASDAS, Ankylosing Spondylitis Disease Activity Score; DMARD, disease-modifying anti-rheumatic drug. [a]n/total number (%).

Most patients without ADA (28/31) against the first anti-TNF drug had clinical inefficacy and detectable serum drug levels just before the switch to the second anti-TNF drug (median (interquartile range): 3,008 (680 to 3,076) ng/ml for Ifx, 3,072 (2,048 to 4,096) ng/ml for Ada, 1,111 (683 to 2,077) ng/ml for Etn), so these patients were considered as having a primary inefficacy. Only three patients treated with Etn without detectable antibodies before switching did not have loss of efficacy and they were switched due only to adverse events. Furthermore, all of 11 patients with ADA against the first anti-TNF treatment had loss of efficacy and undetectable drug levels before switching, so they were classified as having secondary inefficacy related to development of immunogenicity.

Clinical response in relation to immunogenicity

At baseline, no differences in disease activity were observed between patients who did or did not later develop ADA against the first anti-TNF drug (baseline ASDAS first anti-TNF: 3.52 1.03 without ADA vs. 3.14 0.95 with ADA, $p = 0.399$; baseline ASDAS second anti-TNF: 3.36 0.94 without ADA vs. 3.09 0.91 with ADA, $p = 0.466$). Also, there were no differences in clinical activity at baseline to the first and second anti-TNF drugs in patients with and without ADA (without ADA: 3.52 1.03 to the first anti-TNF vs. 3.36 0.94 to the second anti-TNF, $p = 0.383$; with ADA: 3.14 0.95 to the first anti-TNF vs. 3.09 0.91 to the second anti-TNF, $p = 0.922$).

At 6 months after switching, the patients who had developed ADA against the first anti-TNF drug had lower disease activity, as measured by the ASDAS (1.62 0.93 with ADA vs. 2.79 1.01 without ADA, $p = 0.002$) (Figure 1), and more patients had inactive disease (4 out of 11 (36.4%) with ADA vs. 1 out of 31 (3.2%) without ADA, $p = 0.002$) (Figure 2). After 6 months of switching, most patients without ADA against the first anti-TNF drug were classified as being in a high or very high disease activity state by the ASDAS (25 out of 31 (80.6%) without ADA vs. 3 out of 11 (27.3%) with ADA, $p = 0.002$) (Figure 2).

discontinuation [11,17]. Part of this treatment failure can be explained by the development of anti-drug antibodies (ADA) [17-20].

To date, only two studies have been published that correlate the clinical response and immunogenicity to anti-TNF drugs in rheumatoid arthritis (RA) patients who switched to a second anti-TNF drug [21,22]. In these studies, RA patients with ADA against the first anti-TNF drug have been shown to have a better clinical response after switching to a second anti-TNF therapy than patients who did not develop ADA against the first anti-TNF drug [21,22]. Until now, no data have been published about the association between immunogenicity to the first anti-TNF drug and the clinical response after switching to a second anti-TNF drug in SpA patients. In this study, we analysed whether the clinical response to a second anti-TNF drug is conditioned by the development of ADA against the first anti-TNF drug in a group of SpA patients.

Materials and methods

Patients and sera

A total of 42 SpA patients (27 AS, 10 nonradiographic axial SpA, 2 SpA associated with IBD, 2 psoriatic SpA and 1 SpA secondary to reactive arthritis) without previous biological treatment were included. All of these patients had axial involvement and most of them had some peripheral articular manifestation as dactylitis, enthesopathy, monoarthritis and oligoarthritis (28/42 (66.7%) SpA patients: 13 AS, 10 nonradiographic axial SpA, 2 psoriatic SpA, 2 SpA related to IBD and 1 reactive arthritis. The patients were enrolled at the Department of Rheumatology of La Paz University Hospital. This was an ambispective observational study that was approved by the La Paz Hospital Ethics Committee, and all patients provided informed written consent. The retrospective study period covered the years 2005 to 2008, and the prospective study period covered 2009 to 2011. All of the AS patients fulfilled the New York revised criteria for AS [23]. The psoriatic arthritis patients fulfilled the GRAPPA group criteria [24].

All patients received anti-TNF drugs as a first biological treatment (infliximab (Ifx), adalimumab (Ada) and etanercept (Etn)) and later switched to a second anti-TNF drug (Ifx, Ada, Etn and golimumab (Gol)). The selection of all anti-TNF drugs was left to the discretion of the physician, with consideration of patient characteristics, type of disease, and patient preference. Owing to the observational design of the study, no specific criteria for drug withdrawal were required, and the diagnoses of treatment failure and adverse events were based on the judgement of the treating physician. Ifx was administered intravenously at 5 mg/kg at 0, 2 and 6 weeks and every 8 weeks thereafter, and the remaining anti-TNF drugs were administrated subcutaneously (Ada, 40 mg/2 weeks; Etn, 50 mg/week; and Gol, 50 mg/month).

Disease activity was measured by the Ankylosing Spondylitis Disease Activity Score (ASDAS) [25,26] and was assessed at baseline and every 6 months. At the time of inclusion, all patients had evidence of active spinal disease, as indicated by a mean ASDAS of 3.42 1.01. Clinically important improvement was defined as change in ASDAS ≥1.1 [26]. Data related to the clinical activity in the retrospective period were obtained from our database of patients on biological therapy.

Blood samples were collected a maximum of 24 hours before biological drug administration for subcutaneous anti-TNF or just before intravenous infusion for Ifx. Precise timing was required to compare the results because the drug levels in the serum can become undetectable over longer time intervals as a result of normal drug pharmacokinetics rather than the formation of immunocomplexes with ADA. All sera, including those of the retrospective period, were stored at -20 C until the drug and ADA concentrations were measured.

Measurement of drug and anti-drug antibody concentrations

The serum drug concentrations (Ifx, Ada and Etn) were determined by sandwich ELISA, as described previously [27-29]. Serum drug levels were considered positive for Ifx if >10 ng/ml, for Ada if >5 ng/ml and for Etn if >30 ng/ml.

Serum ADA levels (antibodies to Ifx, antibodies to Ada and antibodies to Etn) were detected using a two-site (bridging) ELISA, as previously described [27-29]. The cutoff value for the presence of antibodies to Ifx was established at 50 AU/ml, for antibodies to Ada at 10 AU/ml and for antibodies to Etn at 50 AU/ml.

To determine the cutoff value of each assay, sera from 150 healthy controls and from 100 RA patients without anti-TNF treatment (70% positive for rheumatoid factor) were studied. The mean 6 standard deviations was used to establish cutoff points.

Statistical analysis

The statistical analyses were performed using the Statistical Package for the Social Sciences, version 11.0 (SPSS, Chicago, IL, USA). Descriptive statistics included the mean and standard deviation or the median and interquartile range. Differences in baseline characteristics were assessed using Pearson s chi-square test and Fisher s exact test for ordinal variables and using the Mann-Whitney U test for continuous variables. The continuous data were compared between groups using the Mann-Whitney U test. Statistical significance was calculated using the log-rank test, and $p < 0.05$ was considered significant.

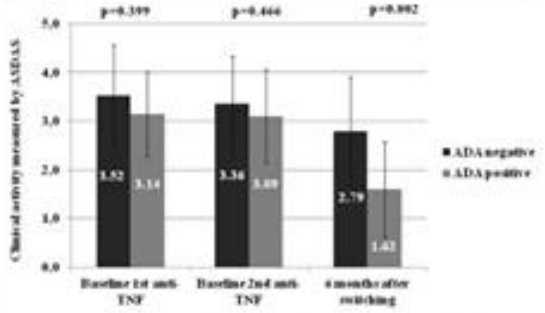

Figure 1 Association between clinical activity (Ankylosing Spondylitis Disease Activity Score) and immunogenicity. Ankylosing Spondylitis Disease Activity Score (ASDAS; mean standard deviation) measured at baseline (first and second anti-TNF drugs) and at 6 months after switching to a second anti-TNF drug in patients who did or did not develop anti-drug antibodies (ADA) against the first anti-TNF drug.

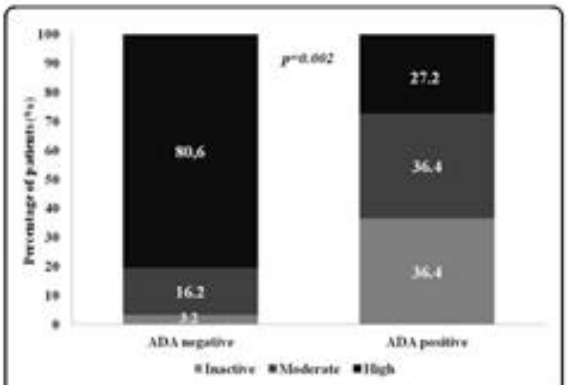

Figure 2 Association between anti-drug antibody status and clinical activity (Ankylosing Spondylitis Disease Activity Score) after switching. Clinical activity classified into inactive (light shading), moderate (medium shading) and high/very high activity (dark shading), according to Ankylosing Spondylitis Disease Activity Score criteria, 6 months after switching to a second anti-TNF drug in patients who had developed anti-drug antibodies (ADA) against the first anti-TNF drug.

At 6 months after switching we observed a greater clinical improvement, as measured by change in ASDAS, in patients with ADA as compared with those without ADA (1.49 1.27 with ADA vs. 0.56 1.01 without ADA, $p = 0.014$) (Figure 3). A total of 13 patients achieved clinically relevant improvement, and clinical improvement was more frequent in patients who had developed ADA (8t out of 11 (72.7%) with ADA vs. 5 out of 31 (16.1%) without ADA, $p = 0.001$). Three out of the five patients without ADA who had an important clinical improvement after switching were treated with Etn as the first anti-TNF drug and the reason for change to a second anti-TNF drug was adverse effects.

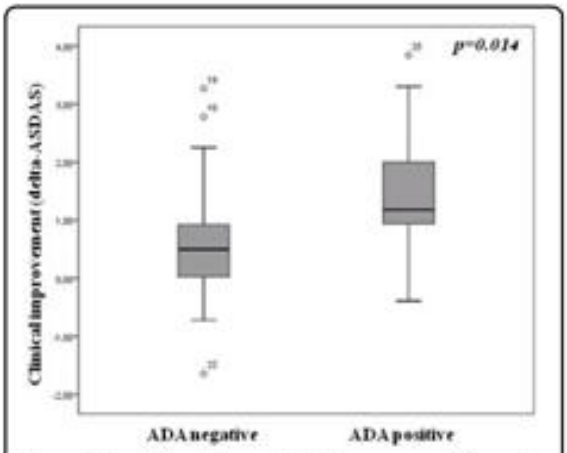

Figure 3 Association between clinical improvement (change in Ankylosing Spondylitis Disease Activity Score) and immunogenicity. Change in Ankylosing Spondylitis Disease Activity Score (delta-ASDAS) measured 6 months after switching in spondyloarthritis patients who presented or not anti-drug antibodies (ADA) against the first anti-TNF. Data shown as interquartile ranges (p75, upper edge; p25, lower edge; p50, midline of the box).

When a subanalysis is performed taking only the group of patients with AS ($n = 27$), we observed that our results in relation to clinical activity and immunogenicity are consistent with those observed analysing all 42 patients. At baseline of the first and second anti-TNF drugs, no differences were seen in clinical activity (ASDAS) between patients with and without ADA (baseline first anti-TNF: 3.61 1.11 without ADA vs. 3.57 0.83 with ADA, $p = 0.929$; baseline second anti-TNF: 3.43 0.77 without ADA vs. 2.87 1.01 with ADA, $p = 0.136$). However, 6 months after switching the clinical activity was lower in patients with previous ADA (2.78 1.05 without ADA vs. 1.38 0.75 with ADA, $p = 0.0.004$).

No differences were observed in clinical activity and clinical improvement between patients treated with a second anti-TNF drug with a mAb or fusion protein (ASDAS after 6 months of switching: 2.47 1.12 with mAb vs. 2.53 1.11 with fusion protein, $p = 0.908$; change in ASDAS after 6 months of switching: 0.73 1.17 with mAb vs. 1.13 1.03 with fusion protein, $p = 0.372$).

Discussion

In this article we studied the role of immunity against a first anti-TNF drug in the short-term response to a second anti-TNF drug in a group of SpA patients. We show that patients in whom drug discontinuation was associated with ADA development achieved a better clinical response at 6 months after switching than patients who had not developed ADA.

Anti-TNF drugs are the only biological therapies available to treat SpA patients with an inadequate response to conventional treatment, and their efficacy has been demonstrated in several randomised placebo-controlled studies [2-9]. However, a number of patients fail to respond or experience adverse events necessitating treatment discontinuation [11,17,20,30,31]. Thus, it is crucial to know what factors predict the treatment response in SpA patients. Different predictors of a favourable response to the first anti-TNF drug have been reported in the literature, including shorter disease duration, younger age, HLA-B27 positivity, a lower Bath Ankylosing Spondylitis Functional Index score, higher C-reactive protein levels, a higher Bath Ankylosing Disease Activity Index score, male sex and the presence of peripheral arthritis and spinal inflammation on magnetic resonance imaging [18,32-39]. These data were not analysed in the present study because we only recruited SpA patients who had discontinued their first anti-TNF drug.

Only a few prospective studies have reported detailed information about switching to a second anti-TNF drug in AS patients [11,40,41]. One recent publication showed that switching to a second anti-TNF drug could benefit some AS patients, and up to one-third of patients achieved a good response [11]. However, disease activity at 3 months after switching was generally worse for switchers on their second anti-TNF drug than for nonswitchers [11]. Similar findings were observed in another study that included 1,250 AS patients, 326 of whom had previously received an anti-TNF drug [37]. In this study, anti-TNF-na ve AS patients achieved greater treatment responses than patients who switched to a second anti-TNF drug [37].

The immunogenicity of biological therapies has been shown to influence secondary inefficacy in rheumatic diseases [17,28,42-52]. The frequency of ADA development in SpA patients varies between different studies (25.5 to 29% for antibodies to Ifx and 31% for antibodies to Ada) [17,19,20,49-51]. Several publications have described the relationship between the development of ADA and the clinical response in SpA patients [17,19,20,52-54]. In previous work conducted by our group, antibodies to Ifx were detected in 25.5% of SpA patients treated with Ifx, and a strong correlation was observed between antibodies to Ifx development and clinical response as measured by the ASDAS [49]. de Vries and colleagues observed that 31% of AS patients treated with Ada developed antibodies to Ada, and most of the patients did not reach an ASAS response after 6 months of treatment [20]. In this study, 26.2% (11/42) of patients who discontinued the first anti-TNF drug had detectable ADA, and most had exhibited a good response to therapy until ADA development. However, the majority of SpA patients without ADA who switched to a new anti-TNF drug never demonstrated clinical improvement, and indeed they typically had a detectable serum drug concentration. These findings may suggest that TNF is not the main cytokine instigating disease activity in these patients or that symptoms in these patients are not related to the inflammatory activity of the disease.

Currently, there are only two reports that relate immunogenicity status to the first anti-TNF drug and its clinical response after switching to a second anti-TNF drug in RA patients [21,22]. Bartelds and colleagues observed that RA patients who had developed antibodies to Ifx against the first anti-TNF drug had no significant differences in clinical improvement after switching (change in Disease Activity Score for 28 joints) when compared with anti-TNF-na ve patients [21]. Nevertheless, switchers without antibodies to Ifx exhibited a significantly lower clinical response than na ve RA patients [21]. Similar findings were reported in a subsequently published study in RA patients (na ve and switchers) treated with Etn [22], which demonstrated that na ve patients and switchers with ADA had a greater clinical response than did patients who switched without ADA [22]. To our knowledge, the present work is the first report in which the influence of immunogenicity against the first anti-TNF drug has been associated with clinical activity after switching in SpA patients. As shown above, clinical improvement was greater in patients who switched after developing ADA, and 73% of patients who achieved clinical improvement after switching had developed ADA to the previous anti-TNF drug.

Our study has several limitations. The number of patients is relatively small because all patients came from the same centre, and SpA patients are less likely than RA patients to discontinue anti-TNF drug [38,41]. However, our results show differences in treatment outcomes that are similar to those described after switching anti-TNF drugs in RA [21,22]. Furthermore, the decision to stop therapy or to change drugs was not standardised because it was based on the decision of the responsible rheumatologist. This may explain why disease activity, although not significantly different at the baseline of the second anti-TNF drug, was slightly lower in the group that developed ADA, because patients are more sensitive to deterioration once they have previously improved, and the switch is performed at lower levels of disease activity. However, it is important to highlight that this is the pattern normally used to determine whether a therapeutic change is required in clinical practise. Finally, different anti-TNF drugs were used in the study, both as the first and the second anti-TNF therapies, which may have influenced the results. However, no differences in the effectiveness of the three most commonly used anti-TNFs have been demonstrated in clinical practice in AS [55], so it is unlikely that this would have affected the results.

Conclusions

Similar to RA, the failure to respond to a first anti-TNF drug due to the development of ADA predicts a better clinical response to a second biological treatment in SpA. The presence of ADA against the first anti-TNF drug is a determining factor for the response to a second anti-TNF drug. The study of the immunogenicity in biological treatment failure may help predict the response to a second biological treatment in SpA.

Abbreviations

Ada: adalimumab; AS: ankylosing spondylitis; ASDAS: Ankylosing Spondylitis Disease Activity Score; ADA: anti-drug antibodies; ELISA: enzyme-linked immunosorbent assay; Etn: etanercept; Gol: golimumab; IBD: inflammatory bowel disease; Ifx: infliximab; mAb: monoclonal antibody RA: rheumatoid arthritis; SpA: spondyloarthritis; TNF: tumour necrosis factor.

Competing interests

AB has received fees from Roche, Schering-Plough, Wyeth, Abbott, BMS and UCB. EM-M is a consultant and a member of speakers' bureaus for Pfizer, MSD, UCB and Abbott. ChP, DP-S and LN have received speaker honoraria from Pfizer. All other authors declare that they have no competing interests.

Authors' contributions

ChP, DP-S, EM-M and AB wrote the article. DP-S, SGC, MTL-C and ChP carried out the data collection and databases. AV, DP, LN, LL, SGC, AB, EM-M and ChP performed the clinical evaluation of patients. JD, SGC and ChP performed the statistical analysis. DP-S and FA performed the laboratory assays. All authors read and approved the final manuscript.

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ARTICLE 8

TITLE: "*Effect of Infliximab Dose Increase in Rheumatoid Arthritis at Different Trough Concentrations: A Cohort Study in Clinical Practice Conditions*"

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PATIENTS AND METHODS

Patients

A retrospective observational study was conducted of RA patients from RA-Paz cohort, who started treatment with Ifx as the first TNFi. The following inclusion criteria were used: RA patients (older than 18years) treated from 2005 to 2011 who exhibited an insufficient clinical response defined as DAS28 >3.2, who received an increase in Ifx dose therapy regimen, and who had serum sample and clinical assessment data during the first year of therapy. A final observation was carried forward for analysis in patients who stopped Ifx treatment within the first year after dose increase. This study was approved by the research ethics committee of the Hospital Universitario La Paz.

Treatment

Initially all patients received standard therapy regimen of Ifx. The dose of Ifx was intensified by rheumatologist criteria and clinical response of patients. Dose escalation could also be combined with increasing doses of DMARDs and/or corticosteroids.

Data Collection and Assessments

Medical history, demographic data, ACPA, and RF were retrospectively retrieved prior to Ifx increase (baseline). DAS28score, EULAR response, and serum concentrations of Ifx and ATI were also retrieved at baseline (T1), after the first Ifx dose increment (T2), and at 6 months (T3), and 12 months (T4). ACPA were measured using ELISA, and RF was assessed using nephelometry.

Infliximab Serum and ATI Concentrations

Serum Ifx concentrations were determined using a capture ELISA Serum ATI levels were assayed using a two-site (bridging) in-house.

Statistical Analysis

Descriptive statistics are provided as the mean, SD, median and interquartile range (IQR).A fixed effects analysis of repeated measures was performed, and Bonferroni correction was used for multiple comparisons. Qualitative variables were compared at different time points between different groups using Fisher's test, and the Bonferroni test was used for multiple comparisons. A regression mixed model for repeated measurements was performed using group (no, low, and high Ifx levels) and time points (T1, T2, T3, andT4) as factors to compare DAS28 and delta-DAS28 between groups. Interactions between factors were calculated as fixed effects, and subjects were calculated as random effects. Pair-wise comparisons were calculated using Bonferroni correction. EULAR was analyzed using a generalized linear model with cumulative logit link function. A significance level of 0.05 was used for statistical testing, and all analyses were performed using SAS9.3 (SAS Institute, Cary, NC, USA). Patients were assigned to one of three serum Ifx groups: no detectable, low (<1.1 μg/mL) or high (≥1.1 μg/mL). These cut- off levels were based on the observation that serum Ifx >1 mg/L (12) was associated with improved disease.

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ORIGINAL RESEARCH
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Effect of infliximab dose increase in rheumatoid arthritis at different trough concentrations: a cohort study in clinical practice conditions

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Background: Evidence supporting treatment intensification in rheumatoid arthritis (RA) is limited and controversial. We explored outcomes of infliximab dose increases and accounted for pre-existing trough levels in patients with active RA.

Methods: This study was a retrospective study of 42 RA patients who received increased infliximab following an insufficient response (DAS28 >3.2). Serum concentrations of infliximab and antibodies to infliximab (ATI) and DAS28 and EULAR clinical response parameters were recorded for 1 year. Analyses were performed in three patient groups that were defined by infliximab serum concentration prior to treatment enhancement: no detectable, low (<1.1 μg/mL) or high (≥1.1 μg/mL) drug levels.

Results: No circulating infliximab was detected in 20 patients (47.6%), but 13 (31%) and 9 (21.4%) patients exhibited low and high levels, respectively. ATI was only detected in patients with no detectable drug levels because the drug interferes with ELISA. DAS28 disease activity globally showed a modest improvement after dose escalation, but this improvement did not persist after 6 and 12 months. Infliximab serum levels increased significantly in the high group ($p = 0.016$), but no increase was achieved in the low and no detectable groups. The three study groups exhibited similar disease activity over time, and no improvement was observed in the non-responder EULAR rates.

Conclusion: These results suggest that the efficacy of an infliximab dose increase is limited, and the response is independent of the infliximab trough serum concentration that is achieved prior to escalation.

Keywords: rheumatoid arthritis, infliximab, dose increase, clinical efficacy

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Introduction

The response to tumor necrosis factor (TNF) inhibitor treatment in chronic inflammatory diseases exhibits great therapeutic variability. Failure to respond to anti-TNF therapy may occur at treatment onset, or it may be secondary to an initial improvement (1, 2). A concentration-dependent effect was described for anti-TNF treatment (3, 4), and inadequate serum trough drug levels is a major

cause for non-responsiveness (3). The development of anti-drug antibodies [antibodies to infliximab (ATI)] is a major source of drug clearance, and it is associated with lower serum drug levels and lack of response (5). However, an optimal therapeutic concentration is not defined, and empirical algorithms for treatment optimization prevail (6). A change in therapeutic target would be appropriate in non-improving patients with high circulating infliximab (Ifx) levels, and switching to another TNF inhibitor was proposed in patients who present no free drug levels and with detectable anti-drug antibodies following treatment. However, increasing the dose of anti-TNF treatment when drug levels are low may achieve a threshold therapeutic concentration (7, 8), and this approach was effective in inflammatory bowel disease (9).

Evidence supporting dose intensification in rheumatoid arthritis (RA) is limited and controversial, and few studies relate dose intensification with pre-increase serum trough drug levels (10–12).

The present study retrospectively analyzed the effect of Ifx dose increase in RA non-responders and accounted for previous drug concentrations to further elucidate the utility of proposed algorithms for patients who do not respond to the first anti-TNF therapy.

Materials and Methods

A retrospective observational study was conducted of RA patients included in the Hospital Universitario La Paz Biologics Registry (Madrid, Spain) who started treatment with Ifx as the first TNF-blocking agent. The following inclusion criteria were used: RA patients (older than 18 years) treated from 2005 to 2011 who exhibited an insufficient clinical response defined as DAS28 >3.2, who received an increase in the dose of Ifx, and who had serum sample and clinical assessment data during the first year of treatment. The period of inclusion ended in 2011 because no increase in Ifx dose was used in our clinic after this time. A final observation was carried forward for analysis in patients who stopped Ifx treatment within the first year after dose increase.

Ifx treatment could be combined with classic disease-modifying anti-rheumatic drugs (DMARDs) and/or corticosteroids. The research ethics committee of the Hospital Universitario La Paz (Madrid, Spain) approved the study, and informed consent was obtained for the storage and future use of serum samples.

Treatment

Patients were initially treated with 3 mg/kg Ifx intravenously at weeks 0, 2, 6, and 14 and every 8 weeks thereafter. The dose of Ifx was increased via the administration of 4, 5, or 6 mg/kg Ifx or a reduction in the administration interval to 7 or 6 weeks, with a maximum dose of 6 mg/kg every 6 weeks. Dose escalation could also be combined with increasing doses of DMARDs and/or corticosteroids.

Data Collection and Assessments

Medical history, demographic data, anti-citrullinated protein antibodies (ACPA), and rheumatoid factor (RF) were retrospectively retrieved prior to Ifx increase (baseline). DAS28 score, EULAR response, and serum concentrations of Ifx and ATI were also retrieved at baseline (T1), after the first Ifx dose increment (T2), and at 6 months (T3), and 12 months (T4). ACPA were measured using ELISA (Eurodiagnostica, Malmo, Sweden), and RF was assessed using nephelometry (Siemens, Marburg, Germany) with cut-off values of 25 and 9 IU/mL. DAS28 was calculated using the erythrocyte sedimentation rate (ESR), and the response to Ifx was evaluated using the European League Against Rheumatism (EULAR) criteria (13).

Infliximab Serum and ATI Concentrations

Serum Ifx concentrations were determined using a capture ELISA as described previously (14), but a biotinylated monoclonal anti-Ifx idiotype antibody (Progenika Bipopharma S.A., Vizcaya, Spain) was used instead of a rabbit antibody to detect Ifx. Serum ATI levels were assayed using a two-site (bridging) in-house ELISA (15) with a cut-off for positivity of 50 arbitrary units (AU)/mL.

Statistical Analysis

Descriptive statistics are provided as the mean, SD, median and interquartile range (IQR). A fixed effects analysis of repeated measures was performed, and Bonferroni correction was used for multiple comparisons. Qualitative variables were compared at different time points and between different groups using Fisher's test, and the Bonferroni test was used for multiple comparisons.

A regression mixed model for repeated measurements was performed using group (no, low, and high IFX levels) and time points (T1, T2, T3, and T4) as factors to compare DAS28 and delta-DAS28 between groups. Interactions between factors were calculated as fixed effects, and subjects were calculated as random effects. Pair-wise comparisons were calculated using Bonferroni correction. EULAR was analyzed using a generalized linear model with cumulative logit link function. A significance level of 0.05 was used for statistical testing, and all analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA).

Patients were assigned to one of three serum Ifx groups: no detectable, low (<1.1 μg/mL) or high (≥1.1 μg/mL). These cut-off levels were based on the observation that serum Ifx >1 mg/L (12) was associated with improved disease (16).

Results

Patient Characteristics

Forty-two patients (37 women) were included in the study. Twenty patients exhibited no detectable free Ifx, 13 patients exhibited Low levels, and 9 patients exhibited High levels. **Table 1** shows the descriptive statistics.

The age of disease onset was significantly lower in patients with no detectable drug levels, and the duration of Ifx treatment was significantly longer for patients with High drug levels. Sixteen patients in the no free Ifx group received an increased Ifx dose, and the treatment interval was reduced in eight patients. Eleven and nine patients in the low and high groups, respectively, received an increased Ifx dose, and the treatment interval was reduced in five and four patients, respectively. Both strategies were used simultaneously in some patients, and these numbers are higher.

TABLE 1 | Demographic and clinical characteristics of patients.

	Total study population ($n = 42$)	No detectable Ifx levels ($n = 20$)	Low Ifx levels ($n = 13$)	High Ifx levels ($n = 9$)
Age at onset (years), mean ± SD	57.1 ± 14.0	49.6 ± 14.5	61.6 ± 10.8	67.4 ± 6.1
Female, *n* (%)	37 (88.1)	19 (95)	9 (69.2)	9 (100)
Disease duration (years), mean ± SD	19.4 ± 10.4	16.3 ± 6.3	17.9 ± 10.1	28.3 ± 13.8
Duration of Ifx treatment (years), median (IQR)	6.2 (1–13)	4.25 (1.23–8.63)	6.25 (4.38–10.75)	8.25 (8.25–10.25)
ACPA-positive, *n* (%)	36 (85.7)	19 (95)	12 (92.3)	5 (55.6)
RF-positive, *n* (%)	35 (83.3)	18 (90)	10 (76.9)	7 (77.8)
Methotrexate therapy, *n* (%)	36 (85.7)	16 (80)	12 (92.3)	8 (88.8)
Methotrexate dose (mg/week), median (IQR)	12.5 (0–25)	15.0 (0–15)	15 (0–20)	10 (0–15)
Other DMARDs, *n* (%)	18 (42.9)	9 (21.4)	4 (9.2)	5 (11.9)
Concomitant use of glucocorticoids, *n* (%)	28 (66.6)	13 (30.9)	8 (19)	7 (16.6)
Prednisone dose (mg/day) before Ifx increase, mean ± SD	6.2 ± 5.2	5.7 ± 6.8	4.2 ± 3.2	7.0 ± 6.4
Prednisone dose (mg/day) at one year, mean ± SD	7.9 ± 6.3	8.7 ± 7.6	7.1 ± 6.3	8.2 ± 5.6
DAS28 at the start Ifx treatment, mean ± SD	5.50 ± 1.20	5.68 ± 1.29	5.03 ± 1.04	5.77 ± 1.12
Baseline DAS28 before Ifx increase, mean ± SD	4.55 ± 1.01	4.91 ± 0.73	3.72 ± 0.90	4.97 ± 1.06
Trough Ifx levels before dose increase (μg/mL), median (IQR)	94.5 (0–10.5)	ND	574 (16–1024)	2112 (1152–10464)
ATI levels before Ifx increase, AU/mL, median (IQR)	0 (0–60000)	1068.5 (377.5–12328.0)	0 (0–0)	ND

Ifx, Infliximab; SD, standard deviation; IQR, interquartile range; ACPA, anti-citrullinated peptide antibodies; RF, rheumatoid factor; DMARDs, disease-modifying anti-rheumatic drugs; DAS28, disease activity score in 28 joints; ATI, anti-infliximab antibodies; AU/mL, arbitrary units per milliliter; ND, not detectable.

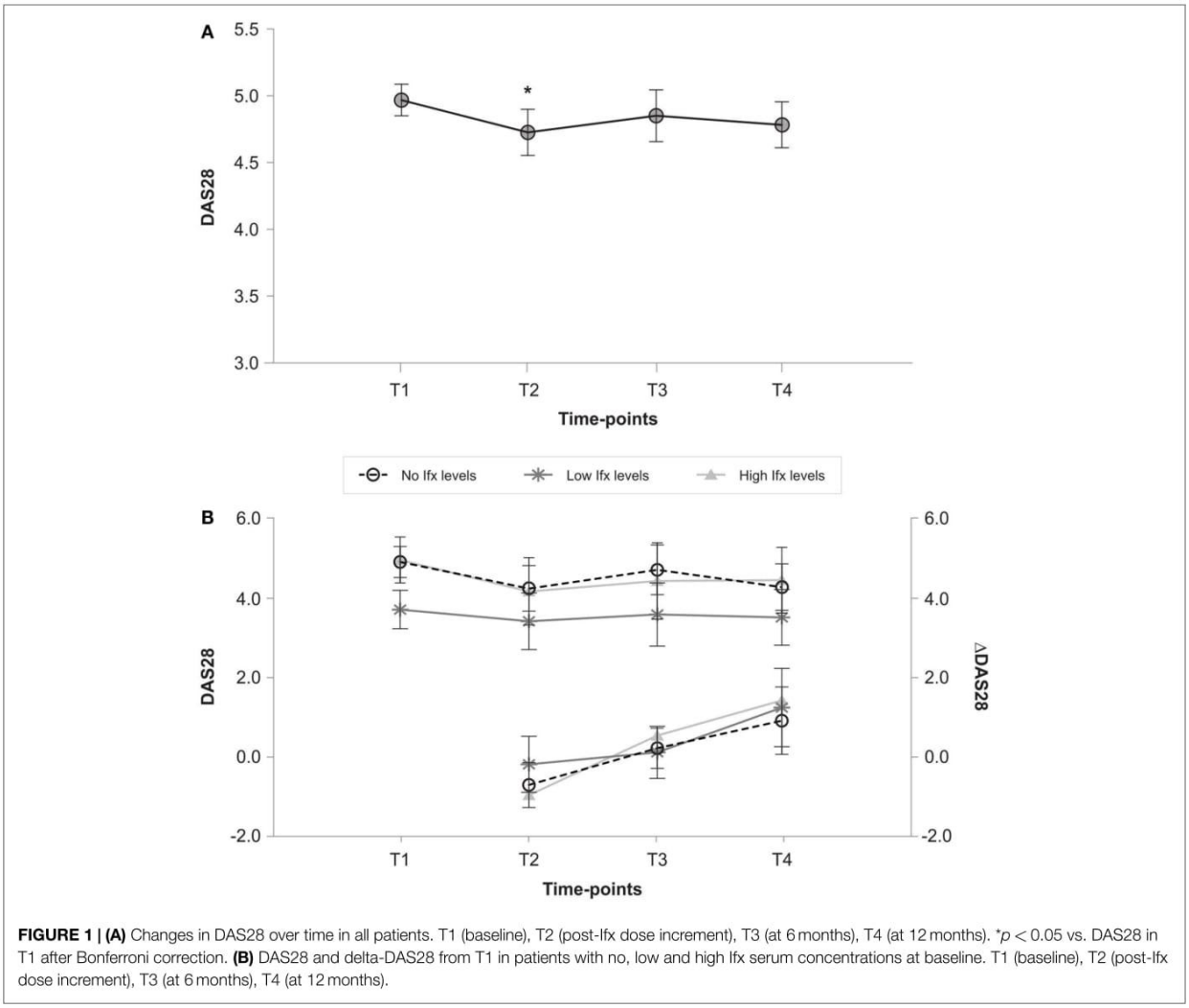

FIGURE 1 | (A) Changes in DAS28 over time in all patients. T1 (baseline), T2 (post-Ifx dose increment), T3 (at 6 months), T4 (at 12 months). *$p < 0.05$ vs. DAS28 in T1 after Bonferroni correction. **(B)** DAS28 and delta-DAS28 from T1 in patients with no, low and high Ifx serum concentrations at baseline. T1 (baseline), T2 (post-Ifx dose increment), T3 (at 6 months), T4 (at 12 months).

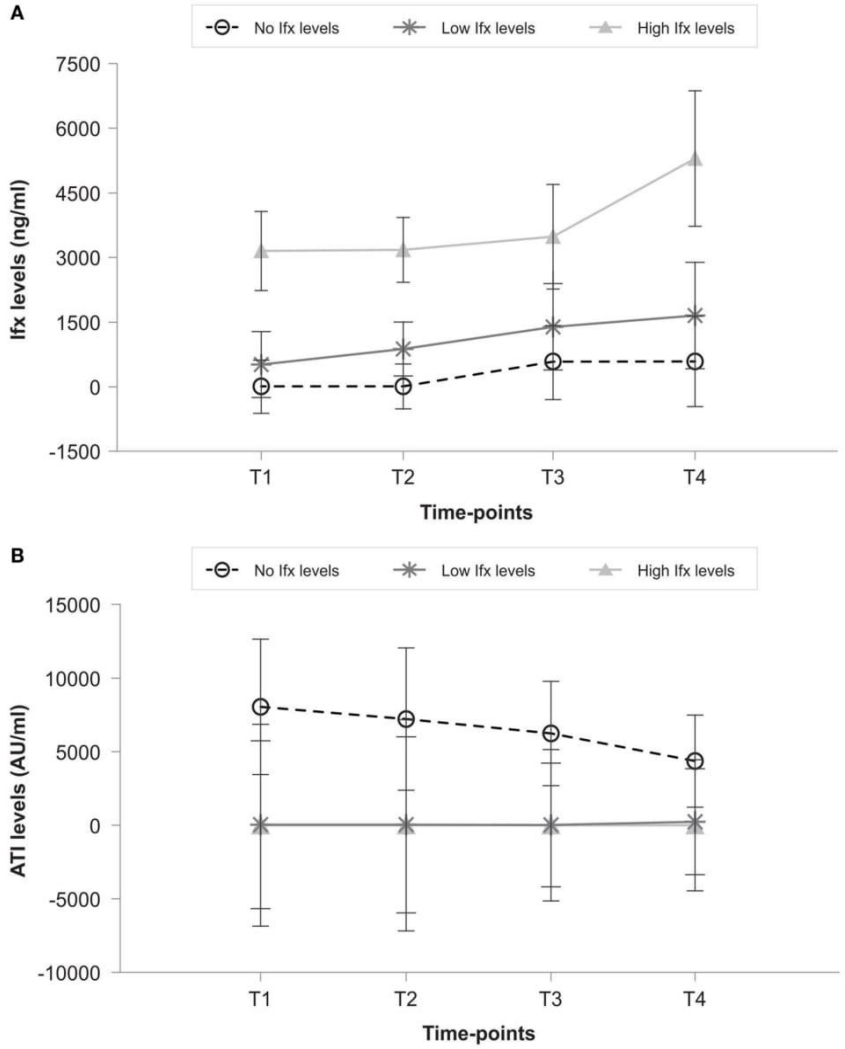

FIGURE 2 | (A) Ifx levels and **(B)** ATI levels in patients with no, low, and high Ifx serum concentrations at baseline. T1 (baseline), T2 (post-Ifx dose increment), T3 (at 6 months), T4 (at 12 months).

Five patients did not complete the year of treatment because of insufficient clinical response (three patients) and side effects (two patients, pneumonia and skin infection).

Effectiveness of Infliximab Dose Increase

Baseline DAS28 for the entire study population (**Figure 1A**) improved immediately after dose increase from baseline (4.55 ± 1.01 vs. 3.95 ± 1.22; $p < 0.05$), but this decrease in DAS28 disappeared at 12 months (3.98 ± 1.22; $p = 0.075$). The change in DAS28 from baseline (delta-DAS28) demonstrated significant disease worsening (from -0.63 ± 1.18 post-increase to 1.17 ± 1.45 after 12 months ($p < 0.001$). **Figure 1B** shows the DAS28 and delta-DAS28 progression for individual patient groups. Basal disease activity was lowest in the low group (3.7 ± 0.9) vs. 4.9 ± 0.7 ($p = 0.001$) and 4.9 ± 1.1 ($p = 0.006$) in the no detectable and high drug level groups, respectively). No significant change in DAS28 was observed in any of the individual patient groups throughout the study. The decrease in disease activity from the time of post-Ifx dose increase was significant in patients with no detectable Ifx levels after 12 months (mean delta-DAS28: 1.0 ± 1.9 vs. -0.7 ± 1.0, $p < 0.05$) and patients with high Ifx levels (mean delta-DAS28: 1.3 ± 1.3 vs. -1.0 ± 1.3; $p < 0.05$).

European League Against Rheumatism response rates in all three patient groups revealed no significant differences at any time point with 11.1, 16.7, and 12.5% of patients in the no, low, and high groups achieving a good response after the first dose increase, and 44.4, 58.3, and 37.5%, respectively, remained non-responders. Good responders after 12 months of enhanced treatment included 25% of no Ifx patients and 0% of low and high Ifx patients, and 50, 77.8, and 71.4% of no, low, and high patients, respectively, exhibited no response.

Infliximab and Anti-Infliximab Antibody Concentrations After Dose Increase

Serum Ifx levels were significantly higher in the high group than the low and no groups at any studied points (**Figure 2A**). Ifx serum levels increased significantly between post-increment (T2) and 12 months (T4) in the high group ($p = 0.017$) but not in the low ($p = 0.97$) or no ($p = 1$) groups. No free Ifx was present at 12 months in 4 of 13 patients (30.7%) in the low group despite an increase in Ifx dose. **Figure 2B** shows that ATI levels in the no group ranged from a basal median of 1068.5 (IQR, 377.5–12328.0) AU/mL to 308.5 (IQR, 0.0–2805.0) AU/mL after 1 year, but it remained 0 AU/mL at all times in the low and high groups. ATI became positive with no free Ifx drug available in 3 of the 13 patients (23.0%) in the low group with previous negative ATI.

Discussion

The therapeutic effect of an Ifx dose increase was analyzed in three populations of active non-remitting RA patients who exhibited no detectable, low and high drug levels. No significant improvement in disease activity or responder rate was observed after 1 year of intensified therapy, independently of pre-existing Ifx serum trough levels.

Our observations are consistent with anti-TNF failure treatment algorithms (7, 8) for two of the populations where a dose increase was ineffective, i.e., patients with high levels of Ifx and no response, and patients with non-detectable circulating Ifx due to the presence of ATI. However, treatment intensification is the recommended strategy in patients with low trough Ifx to achieve therapeutic levels of anti-TNF. Isolated analysis of this population did not reveal significant disease improvement despite dose increases in our study, and no significant increase in serum Ifx levels was achieved. Other studies increased the Ifx dose up to 10 mg/kg and found better results. Therefore, we do not conclude that the outcome would have been different with an increased Ifx dose up to 10 mg/kg. However, the cost to administer this dose is much higher (17).

Antibodies to infliximab were only detected in patients with no circulating Ifx because of drug interference with the method (5). The existence of ATI is highly improbable in patients with high Ifx levels, but the presence of hidden immunogenicity in the low Ifx level population may be partially responsible for low serum drug levels and poor outcomes. No Ifx was detected at 1 year in four patients, and three of these patients expressed ATI. Other non-immune Ifx clearance mechanisms, such as drug binding by immune cells expressing Fyc receptors I, II, and III, (18) naturally occurring anti-mouse antibodies binding infliximab (18, 19), and the "inflammation sink" in which highly expressing TNF tissues bind anti-TNF drug (18), may also contribute to the low circulating Ifx in these patients.

Our study has some limitations, such as the retrospective design, the lack of a pre-determined therapeutic protocol to increase Ifx dose, and the lack of a control group, but it reflects the effect of TNF-blocking agents that are used in daily clinical practice. The observational design of this study was also not appropriate for this type of analysis.

Conclusion

The enhancing of Ifx therapy is costly and it poses the risk of increased adverse events (20), which stresses the importance of exploring Ifx efficacy. Increasing the dose of Ifx to counteract therapeutic response failure was unsuccessful in this study regardless of the circulating drug levels prior to dose escalation.

Author Contributions

CP, DP-S, and AB were involved in the study design, patient selection and data collection and analysis. CP wrote the article. AV, LN, DP, and MB were directly involved in patient management. TJ, MC, and AM-F performed the serum assays and data collection. All authors were involved in the interpretation of the data and the critical review of the manuscript.

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Chapter 3: Tapering or Discontinuation strategies in rheumatic patients

ARTICLE 9: ***"Anti-TNF discontinuation and tapering strategies in patients with axial spondyloarthritis: a systemic literature review"***

ARTICLE 10: ***"Comparing tapering strategy to the standard dosing regimen of TNF inhibitors in rheumatoid patients with low disease activity"***

ARTICLE 11: ***"Comparing tapering strategy to the standard dosing regimen of TNF inhibitors in spondyloarthritis with low disease activity"***

ARTICLE 9

TITLE: "*Anti-TNF discontinuation and tapering strategies in patients with axial spondyloarthritis: a systematic literature review*"

JOURNAL: Rheumatology 2016;55:1188-1194(142)

AUTHORS: Victoria Navarro Compán; **Chamaida Plasencia Rodríguez**; Eugenio de Miguel; Alejandro Balsa; Emilio Martín Mola; Daniel Seoane Mato; Juan D Cañete.

PATIENTS AND METHODS:

Research question and search strategy

A systematic literature review (SLR) was performed using Medline, EMBASE and Cochrane databases in collaboration with an epidemiologist with expertise on SLR methodology. The search included studies published in English, Spanish or French up to 4 September 2014. The research question was formulated according to the Population, Intervention, Comparison, Outcome and Study design (PICOS) method, in which each of the items was defined as specified below. LDA and clinical remission were not pre-defined, but the exact definition used in each study was collected.

The population was patients with axial SpA (axSpA), AS or non radiographic axSpA (nraxSpA), who achieved LDA or clinical remission after receiving a standard dose of anti-TNF therapy. Two intervention strategies were evaluated: discontinuation of anti-TNF therapy or tapering (dose reduction compared with the standard dose) anti-TNF therapy. Comparison was made with maintaining a standard dose of anti-TNF. The outcome was flare or change (increase) on disease activity parameters. As there is no accepted definition for flare in axSpA, this was not pre-defined, but the exact definition employed in each study was also collected. Longitudinal studies with at least 6 months of follow-up since the intervention (strategy) started were included.

Selection of studies

First, titles and abstracts of the retrieved citations were screened to select articles for full-text review. Later, based on the full-text reading of the selected articles, two readers independently decided whether or not to include a specific study for data extraction and, in instances of disagreement, they discussed until a consensus was reached. Inclusion criteria were based on the PICOS components. Exclusion criteria were non-compliance with the definitions established on the PICOS or insufficient data provided by the article to evaluate the objective of the present study.

Data extraction and data summary

Using a systematic extraction data form developed for this specific purpose, both reviewers independently extracted data for each study, including the following: characteristics of the studies (year of publication, design and follow-up period) and patients (total number of patients, demographics and disease characteristics); definition of LDA or clinical remission used in the study; intervention (anti-TNF therapy and dose regimen); and outcome (definition and percentage of patients who achieved the specific outcome). Furthermore, we also evaluated the quality and potential biases of the studies, assigning an overall quality score per study between 0 and 5 points according to the Oxford level of evidence. To summarize the extracted data, the studies selected for data extraction were classified in two groups based on the type of intervention strategy, namely discontinuation or tapering.

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Original article

Anti-TNF discontinuation and tapering strategies in patients with axial spondyloarthritis: a systematic literature review

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Abstract

Objective. The aim was to evaluate whether anti-TNF discontinuation and tapering strategies are efficacious for maintaining remission or low disease activity (LDA) in patients with axial spondyloarthritis.

Methods. A systematic literature review up to September 2014 was performed using Medline, EMBASE and Cochrane databases. Longitudinal studies evaluating the efficacy of discontinuation/tapering of anti-TNF therapy to maintain clinical response achieved after receiving a standard dose of the same drug were included. The results were grouped according to the type of strategy (discontinuation or tapering) evaluated.

Results. Thirteen studies out of 763 retrieved citations were included. Overall, published data are scarce and the level of evidence of the studies is weak. Five studies provided evidence for assessing discontinuation strategy. The frequency of patients developing flare during the follow-up period ranged between 76 and 100%. The median (range) follow-up period was 52 (36–52) weeks and time to flare 16 (6–24) weeks. Additionally, eight studies evaluating tapering strategy were selected. The percentage of patients maintaining LDA or remission was reported in five studies and ranged between 53 and 100%. The remaining three studies reported the mean change in BASDAI and CRP after reducing the anti-TNF dose and did not observe any relevant increase in these parameters.

Conclusion. Published data indicate that a tapering strategy for anti-TNF therapy is successful in maintaining remission or LDA in most patients with axial spondyloarthritis. However, a discontinuation strategy is not recommended because it leads to flare in most cases. Further studies with an appropriate design covering the whole spectrum of the disease are required to confirm these results.

Key words: anti-TNF, axial spondyloarthritis, discontinuation, tapering

CLINICAL SCIENCE

Rheumatology key messages

- Published evidence on discontinuation and tapering strategies in axial spondyloarthritis is scarce and weak.
- Discontinuation of anti-TNF therapy in patients with axial spondyloarthritis leads to flare in most cases.
- Tapering anti-TNF therapy is successful in maintaining low disease activity in most patients with ankylosing spondylitis.

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Introduction

The spectrum of the disease axial spondyloarthritis (axSpA) includes patients with AS and patients with non-radiographic axSpA (nr-axSpA). In both types of patients, anti-TNF therapy has proven efficacy for improving signs and symptoms in randomized controlled trials (RCTs) [1–5].

In contrast, opposite to RA and PsA, anti-TNF therapy has not been shown clearly to inhibit radiographic progression in patients with axSpA. In addition to this, anti-TNF therapy is costly and not exempt from important adverse effects, some of which are dose dependent [6]. Thus, in clinical practice, the question that arises nowadays is whether anti-TNF therapy might be discontinued or tapered in patients with axSpA who achieve the clinical goal without an increase of the disease activity level. The potential benefits of this strategy could be significant, including a substantial reduction of costs and of safety problems. Although some studies have provided some evidence in this regard, this point is still unclear. Based on this, the objective of the present study was to investigate whether discontinuation or tapering strategies of anti-TNF therapy are efficacious for maintaining remission or low disease activity (LDA) in patients with axSpA.

Methods

Research question and search strategy

A systematic literature review (SLR) was performed using Medline, EMBASE and Cochrane databases in collaboration with an epidemiologist with expertise on SLR methodology. The search included studies published in English, Spanish or French up to 4 September 2014. Supplementary data, research strategy section, available at *Rheumatology* Online, shows all the terms used to conduct the search. The research question was formulated according to the Population, Intervention, Comparison, Outcome and Study design (PICOS) method, in which each of the items was defined as specified below. LDA and clinical remission were not pre-defined, but the exact definition used in each study was collected.

The population was patients with axSpA, AS or nr-axSpA, who achieved LDA or clinical remission after receiving a standard dose of anti-TNF therapy.

Two intervention strategies were evaluated: discontinuation of anti-TNF therapy or tapering (dose reduction compared with the standard dose) anti-TNF therapy. Comparison was made with maintaining a standard dose of anti-TNF.

The outcome was flare or change (increase) on disease activity parameters. As there is no accepted definition for flare in axSpA, this was not pre-defined, but the exact definition employed in each study was also collected. Longitudinal studies with at least 6 months of follow-up since the intervention (strategy) started were included.

Selection of studies

First, titles and abstracts of the retrieved citations were screened to select articles for full-text review. Later, based on the full-text reading of the selected articles, two readers (V.N.-C. and C.P.-R.) independently decided whether or not to include a specific study for data extraction and, in instances of disagreement, they discussed until a consensus was reached. Inclusion criteria were based on the PICOS components. Exclusion criteria were non-compliance with the definitions established on the PICOS or insufficient data provided by the article to evaluate the objective of the present study.

Data extraction and data summary

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Results

Characteristics of the studies

A detailed flow chart with the results of the literature search is shown in Fig. 1. Out of the 763 citations retrieved by the search, 33 studies were selected for full-text review. In total, 13 studies were included for data extraction [7–19]; discontinuation strategy was assessed in five studies, whereas tapering strategy was evaluated in eight studies. The reasons for excluding the remaining studies are depicted in supplementary Table S1, available at *Rheumatology* Online.

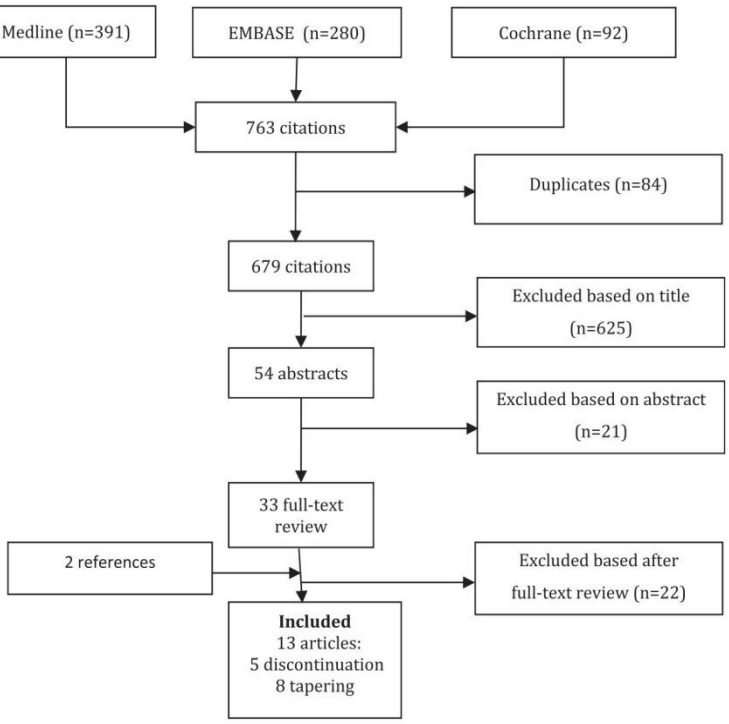
Discontinuation

Five studies [7–11] were included to evaluate the efficacy of an anti-TNF discontinuation strategy for maintaining LDA or remission in patients with axSpA after achieving this state with a standard dose of anti-TNF therapy. Table 1 shows in detail the characteristics and results of these studies. In total, they included 220 patients (84% AS and 16% nr-axSpA). Four of these studies were observational after participation in an RCT, and only one of them was an RCT to evaluate the effect of thalidomide to prevent flare after discontinuation of anti-TNF therapy. The discontinued therapy was etanercept (n = 3), infliximab (n = 1) or adalimumab (n = 1). The median (range) sample size of the studies was 26 (17–111) patients. The median (range) follow-up period after discontinuation was 52 (36–52) weeks. The level of evidence was four in all studies.

Median (range) values for baseline characteristics of patients included in the studies were as follows: age 39 (37–40) years old; 68% (45–77) males; disease duration 8 (3–15) years; 92% (67–100) HLA-B27 positive; and time on anti-TNF therapy before discontinuation 11 (2.5–36) months.

In these studies, patients receiving standard doses of an anti-TNF drug discontinued this therapy and were followed to assess the appearance of flare. The exact

Fig. 1 Flow chart of the studies through review



definition of flare used in each study is depicted in Table 1. In most of them, a flare was defined as BASDAI $\geqslant$ 4 and physician's assessment of disease activity $\geqslant$ 4 or an increase in BASDAI of $\geqslant$2 units. The median (range) percentage of patients developing flare during the follow-up period was 79% (76–100%). Specifically, the percentage and corresponding period for each study were as follows: 100% (within 36 weeks) [7]; 98% (within 48 weeks) [8]; and 76, 79 and 79% (all three within 52 weeks) [9–11]. The median (range) time to flare was 16 (6–24) weeks.

Furthermore, the patients participating in the four observational studies following an RCT were re-treated using the same anti-TNF after flare occurrence. Overall, a similar response to the one achieved at the beginning of the RCT was observed for most clinical parameters of disease activity.

Tapering

Eight studies [12–19] assessed the efficacy of a tapering strategy of anti-TNF therapy after achieving LDA or clinical remission. The characteristics and results of these studies are presented in Table 2. All of them included patients with AS from a single centre, but no study included patients with nr-axSpA. Most of them were observational studies [retrospective (n = 3), prospective (n = 3)] and only two of them were interventional [non-randomized trial (n = 1) and RCT (n = 1)]. The level of evidence was four in all studies except one, in which it was 2b.

Median (range) values for the main characteristics of the studies were as follows: number of patients included in the study 43 (8–136); number of patients on low-dose regimen 21 (8–109); and follow-up period after anti-TNF tapering 12 (6–21) months. The anti-TNF therapy administered was etanercept (n = 5), infliximab (n = 1) or adalimumab/etanercept/infliximab (n = 2). Baseline characteristics of patients included in the studies were as follows: age 41 (35–54) years old; 78% (75–90) males; disease duration 9 (3–13) years; and 89% (75–91) HLA-B27 positive. The time in remission or LDA before reducing the anti-TNF dose was provided in four studies and it was heterogeneous: <3 months (n = 1) and at least 3 (n = 1) or 6 (n = 2) months.

In these studies, patients receiving standard doses of anti-TNF therapy who were in remission (usually defined as BASDAI < 2 and normal CRP) or with LDA (usually defined as BASDAI < 4 and normal CRP) reduced anti-TNF therapy dose according to an established protocol (n = 5) or according to the physician's criterion (n = 3). Dose reduction was most frequently done by increasing the interval between drug administration rather than decreasing the dose of the injection/infusion. The percentage of patients maintaining LDA or remission after reduction of the anti-TNF dose was reported in five out of the eight studies. In detail, these were 67% [18], 75% [19], 53–81% (depending on the anti-TNF therapy) [14], 86% [15] and 100% [17]. The remaining three studies reported

TABLE 1 Characteristics of studies selected for evaluating a discontinuation strategy of anti-TNF therapy in patients with axial spondyloarthritis

Characteristics of the study	Brandt *et al.* [7]	Baraliakos *et al.* [8]	Song *et al.* [9]	Deng *et al.* [10]	Haibel *et al.* [11]
Year	2003	2005	2012	2013	2013
Journal	Arthritis Rheum	Arthritis Res Ther	Ann Rheum Dis	Rheumatol Int	Arthritis Rheum
Design	Prospective, observational after RCT	Prospective, observational after RCT	Prospective, observational after RCT	RCT	Prospective, observational after RCT
Period	2001	2003	2003	–	–
Country	Germany	Germany	International	China	Germany
Number of patients, AS/nr-axSpA	26/0	42/0	6/11	111/0	0/24
Patients who discontinued	26	42	17	111 (thalidomide *vs* SSZ or NSAID)	24
Control group	No	No	No	No	No
Follow-up, weeks	36	52	52	52	52
Characteristics of patients					
Age, years	37	40	34	18–57	38
Male	77	65	71	–	45
Disease duration, years	Mean (S.D.) 14 (9)	Mean (S.D.) 15 (9)	All patients <5 symptom duration	Mean 9	Adalimumab, mean 7; placebo mean 8
HLA-B27+	89	–	94	100	67
Peripheral arthritis	–	–	–	–	30
Synthetic DMARDs	0%	–	–	–	–
Anti-TNF	Etanercept	Infliximab	Etanercept	Etanercept	Adalimumab
Time on anti-TNF, months	3	36	11	2.5	12
Definition to discontinue anti-TNF	⩾20% improvement in BASDAI	All patients (at least, they all had ⩾30% improvement in BASDAI)	ASASpr and remission on MRI	ASAS20 response	ASAS40 response
Outcome					
Flare definition	BASDAI ⩾4 and PhyGV⩾4	BASDAI ⩾4 and PhyGV ⩾4	Increment in BASDAI of 2 units *vs* baseline	Increment in BASDAI of 2 units or 80% of BASDAI prior to treatment	Loss of ASAS40 response *vs* baseline
Number patients with flare	75% at 12 weeks, 100% at 36 weeks	24% at 12 weeks, 98% at 48 weeks	76% at week 52	79% at week 52	79% at week 52
Time to flare, weeks	Mean (S.D.) 6.2 (3.0)	Mean (S.D.) 17 (8)	Mean 24	Mean (S.D.) 14 (9)	Mean (S.D.) 15 (5.5)
Predictor of flare	–	No ASASpr, high BASDAI or high CRP at discontinuation	–	CRP, PtGV, spinal inflammation	Not found
Response after re-treatment	58% BASDAI 50 31% ASAS after 54 weeks	Similar to initial (BASDAI 6.1 at reinfusion *vs* 2.9 after 12 weeks)	Similar to initial except for ASASpr that was less frequently achieved	–	ASAS40 was achieved by 63% after 1 year and 74% after 2 years
Level of evidence					
Oxford level of evidence	4	4	4	4	4

ASASpr: partial response according to Assessment of SpondyloArthritis international Society; nr-axSpA: non-radiographic axial spondyloarthritis; PhyGV: physician's global assessment of disease activity; PtGV: patient's global assessment of disease activity; RCT: randomized controlled trial.

TABLE 2 Characteristics of studies selected for evaluating tapering strategy of anti-TNF therapy in patients with axial spondyloarthritis

Characteristics of the study	Lee *et al.* [12]	Navarro-Compán *et al.* [13]	Paccou *et al.* [14]	Cantini *et al.* [15]	Mörck *et al.* [16]	Borrás-Blasco *et al.* [17]	De Stefano *et al.* [18]	Závada *et al.* [19]
Year	2010	2011	2012	2013	2013	2014	2014	2016
Journal	Clin Rheumatol	Clin Rheumatol	J Rheumatol	Biologics: Targets and Therapy	Mediators of Inflammation	Expert Opin Biol Ther	Clin Rheumatol	Ann Rheum Dis
Design	Retrospective	Prospective	Retrospective	RCT	Clinical trial	Retrospective	Prospective	Prospective
Period	2004–09	2003–10	2001–10	2005–09	2003–06	2003–06	2007–10	2007–13
Country	Korea	Spain	France	Italy	Sweden	Spain	Italy	Czech Republic
Total number of patients	109	51	65 (49)	43	19	8	21	136
Patients with tapering	109	16	49	22	19	8	21	53
Control group	No	35	No	21	No	–	–	83
Follow-up, months	21	At least 6 mean 26	At least 6 mean 30	21	12	6	9	12
Characteristics of patients								
Age, years	35	43	45	37	40	–	44	41
Male (%)	90	87	79	79	74	–	76	75
Disease duration, years	9	8	14	13	8	–	3	9
HLA-B27 + (%)	91	87	80	–	80	–	87	75
Peripheral arthritis	–	19	–	–	–	–	15	34
Synthetic DMARDs (%)	94	31	10	–	100	–	–	11
Anti-TNF	Etanercept	Etanercept	Adalimumab (5), etanercept (17), infliximab (25)	Etanercept	Infliximab	Etanercept	Etanercept	Adalimumab, etanercept or infliximab
Time on anti-TNF, months		17 (12)			12	32 (13)	3	35
Tapering strategy	Protocol	Physician	Physician	Protocol	Protocol	Protocol	Protocol	Physician
Outcome								
Remision or LDA	–	BASDAI <4 and PCR 0	BASDAI <2, no peripheral symptoms and PCR[a]	BASDAI <4, no peripheral symptoms and CRP[a]	–	BASDAI <2	ASASpr	BASDAI <4
Time in remission after tapering, months	–	>6	⩾3	–	–	–	<3	⩾6, mean 27
Remission maintenance (%)	–	–	At month 12: 80% adalimumab 53% etanercept 81% infliximab	86	–	100	67	75
Most frequent regimen	Increasing interval (25 mg/ 12 days)	25 mg/week	40 mg/3 weeks 25 mg/week 5 mg/kg/9 weeks	50 mg/2 weeks	3 mg/kg/8 weeks	25 mg/week	25 mg/2 weeks	Increasing interval (regimen not specified)
Before tapering								
BASDAI	2.3	1.6	–	–	2.1	2.1	–	–
CRP, mg/l	0.06	1.0	–	–	8	–	–	–
After tapering			–	–			–	–
BASDAI	0.6	1.4	–	–	3.2	2.0	–	–
CRP, mg/l	0.06	1.3	–	–	8	–	–	–
Level of evidence								
Oxford level of evidence	4	4	4	2b	4	4	4	4

[a]Value within normal range. ASASpr: partial response according to Assessment of SpondyloArthritis international Society; RCT: randomized controlled trial.

the mean change in disease activity measures after reducing anti-TNF therapy [12, 13, 16]. The mean BASDAI in these studies before reduction of the anti-TNF dose was 2.3, 1.6 and 2.1, and at the end of the study this was 0.6, 1.4 and 3.2, respectively. Mean CRP (in milligrams per litre) before reduction of the anti-TNF dose was 0.06, 1.0 and 8, and at the end of the study this was 0.06, 1.3 and 8, respectively.

Discussion

This SLR summarizes the published evidence on the use of discontinuation or tapering strategies of anti-TNF therapy in patients with axSpA after achieving clinical remission or LDA with a standard dose of the same drug. The findings for a discontinuation strategy are consistent. Published data indicate that discontinuation of anti-TNF therapy in patients with axSpA leads to the appearance of flare within a few months in most cases. Therefore, despite the possible benefits, the use of this strategy is not recommended. However, if for any specific reason, such as surgery or pregnancy, discontinuation is required in a patient with axSpA, published evidence also indicates that the probability of achieving a similar response after reinitiating anti-TNF therapy is very high.

Furthermore, published data also suggest that a tapering strategy may be efficacious to maintain remission or LDA in most patients with axSpA. Although the comparison with other rheumatic diseases is difficult, the frequency of flare after implementation of a tapering strategy seems to be lower in patients with axSpA than in patients with RA [20]. The implementation of this strategy in clinical practice would have a great impact, such as reducing possible adverse effects related to anti-TNF therapy and, especially, saving costs. Importantly, however, not all patients who switched to a tapering strategy remained at the same disease activity status. The identification of those patients who have less probability of success after implementation of the tapering strategy is therefore essential, but so far no predictors have been clearly identified. Among possible predictors, it seems that the time under remission before tapering anti-TNF therapy as well as the absence of peripheral and extra-articular manifestations are associated with a better outcome. In this sense, the use of concomitant DMARDs could also help to maintain remission after implementation of an anti-TNF tapering strategy, but this possible beneficial effect of adding a DMARD to an anti-TNF therapy in patients with axSpA has not been demonstrated and needs further investigation. In addition to this, the consequences of a tapering strategy on long-term outcomes, such as function, mobility, quality of life and cardiovascular mortality, also need to be evaluated.

To our knowledge, this is the first SLR that has examined the efficacy of discontinuation and tapering of anti-TNF therapy in patients with axSpA to maintain remission or LDA after this has been achieved with a standard dose. Nevertheless, this SLR has important limitations that should be considered when interpreting its results. First, published studies evaluating this topic are limited and included a small sample size of patients, mainly attending a specific centre. Second, the overall quality of the studies included is poor. None of the studies had a proper design (non-inferiority RCT) to provide a clear answer to the research question. Third, no established definition for clinical remission, LDA and flare has been used in patients with axSpA [21]. As a consequence, the definitions used in the studies for these outcomes are heterogeneous, which makes the comparison between studies and the interpretation of the SLR results difficult. In addition, most of the studies included only patients with AS and established disease, and only one of them (evaluating a discontinuation strategy) included patients with nr-axSpA. Also, it is necessary to remark that the inclusion criteria used by most of the studies were the modified New York criteria or the European Spondyloarthropathy Study Group criteria, which may contain patients with predominantly peripheral disease. Therefore, the extrapolation of these results to all patients covered by the whole spectrum of the disease axSpA according to the new Assessment of SpondyloArthritis international Society remains to be established.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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ARTICLE 10

TITLE: ***"Comparing a tapering strategy to the standard dosing regimen of TNF inhibitors in rheumatoid arthritis patients with low disease activity"***

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PATIENTS AN METHODS:

Patients, clinical assessment and therapy regimen

In this retrospective observational study two RA cohorts on TNFi were analyzed: a cohort from Spain under a tapering strategy (tapering group: TG) and a cohort from the Netherlands on standard therapy regimen (control group: CG). A total of 493 RA patients (233 from Spain and 260 from the Netherlands) were under TNFi (Ifx, Ada and Etn) but only 144 patients fulfilled the inclusion criteria for this study (67 patients from Spain and 77 patients from the Netherlands). All the selected RA patients fulfilled the1987 ACR revised criteria for RA, signed an inform consent before starting TNFi.

Consecutive RA patients treated with TNFi from Spain, who were under a tapering strategy with low disease activity or remission (DAS28<3.2) for at least 6 months before starting the tapering, were selected. Next, consecutive RA patients treated with TNFi from the Netherlands and in low disease activity or remission for at least 6 months were chosen. Later, both cohorts were matched according to several demographic, serological and clinical characteristics to ensure that both groups were similar at baseline and at visit 1(before starting the tapering strategy. RA patients who did not fulfill these requirements were excluded from the study to avoid misinterpretations using heterogeneous cohorts.

The tapering strategy included a progressive interval prolongation: the interval administration in Ifx and Ada was prolonged 1 week every time and in Etn was delayed 3 days every time as long as the physician decided that the interval of administration could be modified based on clinical and serological markers. The CG remained on the standard dose of biologic therapy throughout the study. Initially, Ifx was administered intravenously at 3 mg/kg at 0, 2 and 6 weeks and every 8 weeks thereafter, and the remaining TNFi were administered subcutaneously (Ada: 40 mg/2 weeks and Etn: 50 mg/week). Clinical activity was measured using DAS28 at different time points: visit 0 (just prior to starting the TNFi), visit1 (before beginning the taper in the TG and at least 6 months with low disease activity in the TG and CG), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1), visit 4 (the last visit available after visit 1) and visit flare (the visit with the worst flare between visit 1 and visit 4). Flares were registered between visit 1 and visit 4; a flare was defined as a DAS28 $\geq$3.2 and delta-DAS<0.6 comparing to DAS at visit 1 on at least one occasion. If a flare occurred in the TG, the interval of biological drugs could be shortened to regain low disease activity and also prednisone, non-biologic DMARDs and/or NSAID could be intensified. When a flare was registered in the CG, intensification in prednisone, non-biologic DMARDs and/or NSAID treatment was used to regain control over the disease activity.

Serum samples and assays to measure drug and ADA levels

The serum drug concentrations (Ifx, Ada and Etn) were determined by in house capture enzyme-linked immunosorbent assay (ELISA). The radioimmunoassay (RIA) by Sanquin Diagnostic Services (Amsterdam, the Netherlands) was performed to detect ADA in patients treated with Ifx and Ada. Both measurements, the drug and ADA levels, were measured in most patients of the TG in the different time studied points. In the CG from the Netherlands, only ADA levels were measured. In general, drug and ADA levels were measured in 65 out of 67 (97%) patients in the TG and ADA levels were tested in 71 out of 77 (92%) patients in the CG.

Statistical analysis

First, descriptive analyses were performed for the demographic and clinical variables. The results are shown as means and standard deviation (SD) for continuous variables and relative frequencies for categorical variables. The frequency data were compared using the Pearson $\chi 2$ and Fisher exact tests. The continuous data were compared between groups using the Mann- Whitney U non-parametric test. Later, the associations between the independent variables and the outcomes were investigated using univariate logistic regression model. Estimates for these associations are shown as standardized linear coefficient. SPSS 20.0 software was employed for the analyses and *p* values less than 0.05 were considered statistically significant.

Comparing a tapering strategy to the standard dosing regimen of TNF inhibitors in rheumatoid arthritis patients with low disease activity

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Abstract

Objective

The aim of this study is to compare clinical outcomes, incidence of flares and administered drug reduction between rheumatoid arthritis (RA) patients under TNF inhibitors (TNFi) tapering strategy and RA patients on standard regimen.

Methods

and the control group with standard therapy regimen (CG: 77 pts from the Netherlands). DAS28 was measured at different time points: visit 0 (prior starting TNFi), visit 1 (prior to start tapering in TG and with DAS28<3.2 in TG and CG), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1), visit 4 (the last visit available after visit 1) and visit-flare (visit with the worst flare between visit 1 and visit 4).

Results

Despite the reduction of administered drug at visit 4 in the TG (interval elongation of 32.8% in infliximab, 52.9%

2.7±0.9 in TG vs. 2.5±1 in CG, p=0.1). No differences were seen in the number of patients with flares [26/67 (38.9%) in the TG vs. 30/77 (39%) in the CG, p=0.324] and only nineteen out of 136 patients (14%) had anti-drug antibodies at the end of the study.

Conclusion

not worse than patients on the standard regimen.

Key words

rheumatoid arthritis, TNF inhibitors, treatment, clinical outcome, tapering strategy

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The other co-authors have declared no competing interests.

Introduction

The use of tumour necrosis factor inhibitors (TNFi) has improved the control of disease activity in rheumatoid arthritis (RA) patients (1-7), but the medical costs have increased in recent years (8-12). In addition, higher doses of biologic agents may be associated with increased side effects (13). Considering these findings, it would be interesting to study the effect of TNFi dose reduction in RA patients. The EULAR recommendations suggest that tapering of a biologics can be considered in RA patients who are in remission (14).

Some publications about discontinuation and dose titration of biologics in RA patients have emerged (15-28). In most studies conducted in patients with early RA on TNFi therapy, TNFi discontinuation has been observed to be feasible (17-20). However, the discontinuation strategy is more controversial in patients with established RA (21-25, 27). In recent years, it has been shown that TNFi dose titration in patients with inactive disease is a feasible strategy in patients with long standing RA (15, 16, 26, 28), though studies with long-term follow-up are lacking.

An important concern of clinicians about the tapering strategy is the appearance of flares and inefficacy in RA patients who previously had inactive disease treated with the standard regimen. Sparse evidence regarding these concerns is available, however, most publications have shown that restarting a TNFi after discontinuation again resulted in good control of disease activity (22-24). Nevertheless, new studies with longer follow-up periods are required about tapering strategy in patients with RA.

The association between drug levels and clinical response has been demonstrated previously in RA patients treated with TNFi (29-34). Low drug levels are correlated with a poor clinical response and poor drug survival (30-33). Some recent studies in RA patients treated with TNFi defined the optimal drug levels to achieve a good clinical response (29, 34). However, the optimal drug levels to maintain remission in patients with inactive disease remain unknown.

Over the last several years, in our hospital, there has been a tendency toward using a tapering strategy together with drug level monitoring in patients at least in low disease activity. Conversely, in the Netherlands, the label dose is maintained even when a good clinical response has been registered in RA patients. Our main objectives were to compare the long term clinical disease activity, incidence of flares and incidence of anti-drug antibodies (ADA) at the end of the study between RA patients under a tapering strategy versus RA patients on standard dose. Our secondary targets were to analyse, only in the RA tapering group, the change on serum drug levels (infliximab, adalimumab or etanercept) during the study and predictors associated with good response to tapering.

Material and methods

Patients, clinical assessment and therapy regimen

In this retrospective observational study two RA cohorts on TNFi were analysed: a cohort from Spain under a tapering strategy (tapering group:TG) and a cohort from the Netherlands on standard therapy regimen (control group: CG). A total of 493 RA patients (233 from Spain and 260 from the Netherlands) were under TNFi (infliximab, adalimumab and etanercept) but only 144 patients fulfilled the inclusion criteria for this study (67 patients from Spain and 77 patients from the Netherlands) (see flowchart in Supplementary file).

All the selected RA patients fulfilled the 1987 ACR revised criteria for RA (35), signed an inform consent before starting TNFi and were informed about the tapering strategy in the Spanish cohort. Consecutive RA patients treated with TNFi from Spain, who were under a tapering strategy with low disease activity or remission (DAS28<3.2) for at least 6 months before starting the tapering, were selected. Next, consecutive RA patients treated with TNFi from the Netherlands and in low disease activity or remission for at least 6 months were chosen. Later, both cohorts were matched according to several demographic, serological and clinical characteristics to ensure that both groups were similar (age, gender, disease duration, presence of positive for RF and ACPA,

the disease activity (DAS28) at baseline and at visit 1(before starting the tapering strategy), duration of inactive disease prior to visit 1 and the time of follow-up between visit 1 and visit 4). All included patients were Caucasian. RA patients who did not fulfill these requirements were excluded from the study to avoid misinterpretations using heterogeneous cohorts (Supplementary file).

The tapering strategy included a progressive interval prolongation: the interval administration in infliximab and adalimumab was prolonged 1 week every time and in etanercept was delayed 3 days every time as long as the physician decided that the interval of administration could be modified based on clinical and serological markers. The CG remained on the standard dose of biologic therapy throughout the study. Initially, infliximab was administered intravenously at 3 mg/kg at 0, 2 and 6 weeks and every 8 weeks thereafter, and the remaining TNFi were administered subcutaneously (adalimumab: 40 mg/2 weeks and etanercept: 50 mg/week).

Clinical activity was measured using the Disease Activity Score of 28 joints (DAS28) at different time points: visit 0 (just prior to starting the TNFi), visit 1 (before beginning the taper in the TG and at least 6 months with low disease activity in the TG and CG), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1), visit 4 (the last visit available after visit 1) and visit flare (the visit with the worst flare between visit 1 and visit 4).

Flares were registered between visit 1 and visit 4; a flare was defined as a DAS28 ≥3.2 and delta-DAS<0.6 comparing to DAS at visit 1 on at least one occasion. If a flare occurred in the TG, the interval of biological drugs could be shortened to regain low disease activity and also prednisone, non-biologic DMARDs and/or NSAID could be intensified. When a flare was registered in the CG, intensification in prednisone, non-biologic DMARDs and/or NSAID treatment was used to regain control over the disease activity.

Serum samples and assays to measure drug and ADA levels

Blood samples were collected within a maximum of 24 hours before biologic drug administration. The serum drug concentrations (infliximab, adalimumab and etanercept) were determined by in house capture enzyme-linked immunosorbent assay (ELISA) using antisera from Progenika (Derio, Vizcaya, Spain) as described previously (30, 36, 37). The radioimmunoassay (RIA) by Sanquin Diagnostic Services (Amsterdam, the Netherlands) was performed to detect ADA in patients treated with infliximab and adalimumab as previously described (36, 38, 39).

Both measurements, the drug and ADA levels, were measured in most patients of the TG in the different time studied points. In the CG from the Netherlands, only ADA levels were measured. In general, drug and ADA levels were measured in 65 out of 67 (97%) patients in the TG and ADA levels were tested in 71 out of 77 (92%) patients in the CG.

Statistical analysis

First, descriptive analyses were performed for the demographic and clinical variables. The results are shown as means and standard deviation (SD) for continuous variables and relative frequencies for categorical variables. The frequency data were compared using the Pearson χ^2 and Fisher exact tests. The continuous data were compared between groups using the Mann-Whitney U non-parametric test. Later, the associations between the independent variables and the outcomes were investigated using univariate logistic regression model. Estimates for these associations are shown as standardised linear coefficient. SPSS 20.0 software was employed for the analyses and *p*-values less than 0.05 were considered statistically significant.

Table I. Demographic characteristics of 144 rheumatoid arthritis patients.

RA* patients n=144 patients	TG* n=67	CG* n=77	*p*
Female, no (%)	55 (82%)	58 (75%)	*0.33*
Age (years), mean ± SD	58.9 ± 13.9	58.5 ± 10.3	*0.75*
Disease duration (years), mean ± SD	16.5 ± 7.2	17.5 ± 7.8	*0.5*
RF*, n/N(%)	52/67 (78%)	58/75 (77%)	*0.97*
ACPA*, n/N(%)	48/67 (72%)	49/62 (79%)	*0.33*
Baseline DAS28*, mean ± SD*	4.9 ± 1	4.8 ± 0.9	*0.56*
Baseline CRP*, mean ± SD mg/l	10.2 ± 13.1	11.1 ± 12.7	*0.63*
Prior biological use, no (%)	8 (12%)	16 (21%)	*0.16*
Duration of low disease activity prior visit 1, mean ± SD (years)	1.1 ± 0.9	0.9 ± 0.5	*0.42*
Duration of follow-up between visit 1-visit 4, m ± SD (years)	2.4 ± 1.2	2.4 ± 0.9	*0.33*
Baseline co-therapy			
Methotrexate only (MTX)	25 (37%)	57 (74%)	*<0.001*
Other DMARDs only (OD)	13 (19%)	1 (1%)	*<0.001*
MTX+OD	21 (32%)	13 (17%)	*0.042*
TNFi monotherapy	8 (12%)	6 (8%)	*0.402*

*RA: rheumatoid arthritis; TG: Tapering group; CG: Control group; RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibodies; DAS28: disease activity score; CRP: C-reactive protein; SD: standard desviation.

The frequency data (sex, RF, ACPA, prior biological use and baseline co-therapy) were compared using the Pearson χ^2 and Fisher exact tests. The continuous data (age, disease duration, baseline DAS28, baseline CRP, duration of low disease activity and duration of follow-up) were compared between groups using the Mann-Whitney U non-parametric test (*p*-values <0.05 were considered as statistically significant).

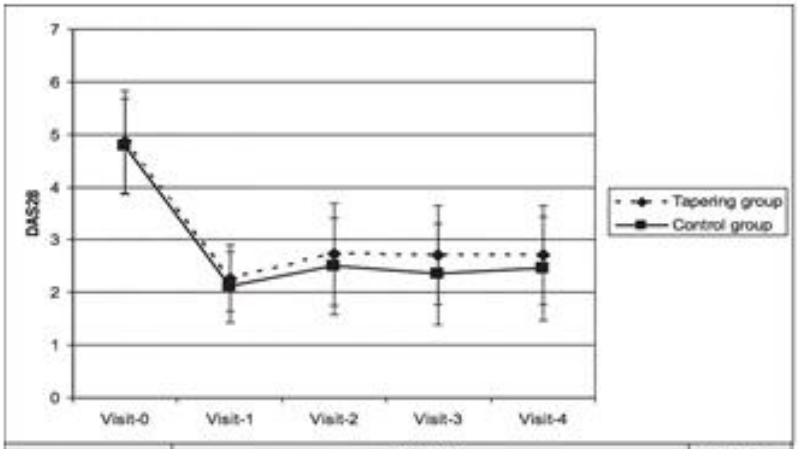
Results

Patient characteristics

A total of 144 RA patients receiving TNFi were enrolled in the study (67 patients in the TG: 23 on infliximab, 23 on adalimumab and 21 on etanercept; 77 patients in the CG: 22 on infliximab, 27 on adalimumab and 28 on etanercept). The patients demographic characteristics are summarised in Table I. No differences in both groups were observed in patients treated in monotherapy with the TNFi, however, in the TG the concomitant therapy with methotrexate plus other DMARDs and other DMARDs only was more used than in the CG.

Clinical response throughout the study

The clinical course measured by the DAS28 was similar in both groups during this study (Fig. 1). In subgroup



	DAS28*		p values
	TG	CG	
Visit 0	4.9 ± 1	4.7± 1	0.6
Visit 1	2.3 ± 0.6	2.1± 0.7	0.09
Visit 2	2.7 ± 1	2.5 ± 1	0.4
Visit 3	2.7± 1	2.4 ± 1	0.09
Visit 4	2.7±0.9	2.5 ± 1	0.1

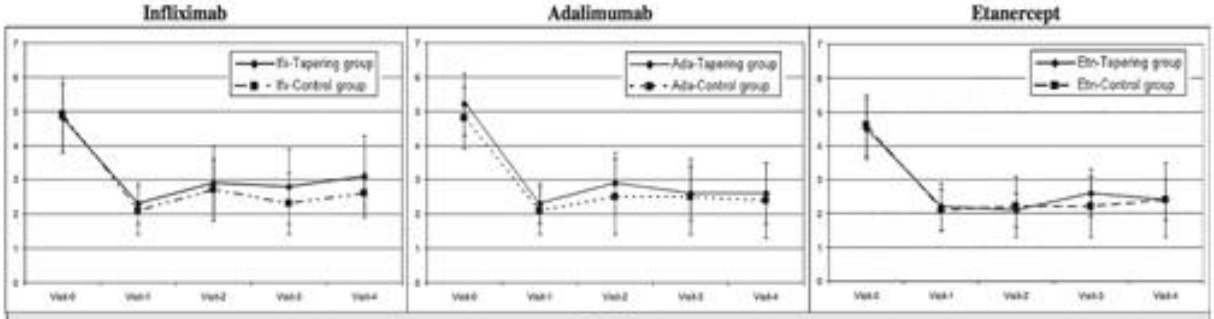
*TG= Tapering group, CG= Control group, DAS28= disease activity score

Fig. 1. Comparison of the clinical activity (DAS28) between tapering and control groups. The clinical evolution was measured by DAS28 (mean ±SD) and compared between TG and CG using the Mann-Whitney U non-parametric test (p-values <0.05 were considered as statistically significant) at different time points during the study: visit 0 (prior starting TNFi), visit 1(pre-tapering), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1) and visit 4 (last visit available after visit 1).

analyses to compare the clinical activity between the different TNFi, no significant differences were observed (Fig. 2). In general, most RA patients still had at least low disease activity at the end of the study [at visit 4: 58/67 (87%) in TG versus 62/77 (81%) in CG, p=0.331], including in a sub-analysis for each TNFi separately [infliximab:17/23 (74%) in TG *vs.* 18/22 (82%) in CG, p=0.524; adalimumab: 21/23 (91%) in TG *vs.* 22/27 (82%) in CG, p=0.318; etanercept: 20/21 (95%) in TG *vs.* 22/28 (79%), p=0.099].

Flares during the study

Fifty six (39%) RA patients experienced a flare during this study [26/67 (39%) in the TG *vs.* 30/77 (39%) in the CG, p=0.324]. No differences were observed in the number of flares between groups (1.8±0.8 in the TG *vs.* 1.7±0.7 in the CG, p=0.575) or in the time to appear the first flare after visit 1 (1.3±0.8 years in the TG *vs.* 1.4±0.7 years in the CG, p=0.580). Table II shows the proportion of patients with flares, the number of flares and the time to the first flare for patients of the TG and CG



	DAS28								
	Infliximab			Adalimumab			Etanercept		
	TG*	CG*	p	TG	CG	p	TG	CG	p
Visit 0	4.8 ± 1	4.9 ± 1.1	0.8	5.2 ± 0.9	4.8 ± 0.9	0.1	4.5 ± 0.9	4.6 ± 0.9	0.5
Visit 1	2.3 ± 0.6	2.1 ± 0.7	0.4	2.3 ± 0.6	2.1 ± 0.7	0.3	2.2 ± 0.7	2.1 ± 0.6	0.5
Visit 2	2.9 ± 1.1	2.7 ± 0.9	0.9	2.9 ± 0.9	2.5 ± 1.1	0.2	2.1 ± 0.5	2.2 ± 0.9	0.4
Visit 3	2.8 ± 1.1	2.3 ± 0.9	0.3	2.6 ± 0.8	2.5 ± 1.1	0.6	2.6 ± 0.7	2.2 ± 0.9	0.3
Visit 4	3.1 ± 1.2	2.6 ± 0.6	0.25	2.6 ± 0.9	2.4 ± 1.1	0.3	2.4 ± 0.6	2.4 ± 1.1	0.5

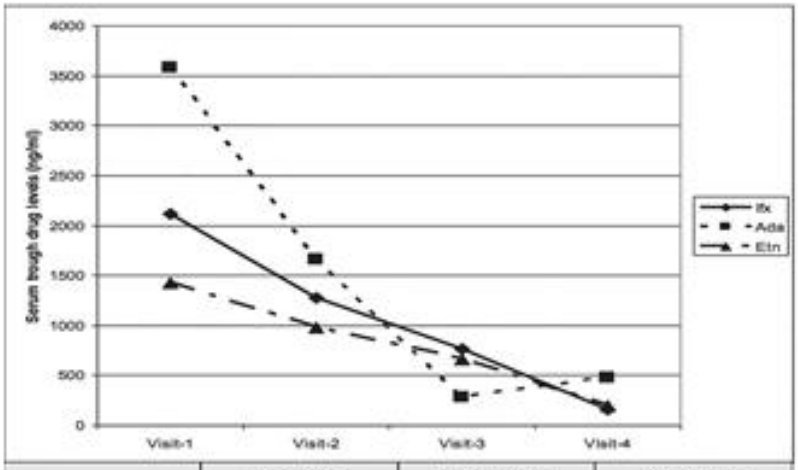
*TG= Tapering group, CG= Control group, Ifx=infliximab, Ada=adalimumab, Etn=etanercept

Fig. 2. Comparison of clinical activity (DAS28) between in tapering and control groups in each TNFi. The clinical activity was measured by DAS28 (mean ±SD, represented in X-axis) and compared between TG and CG using the Mann-Whitney U non-parametric test (p-values <0.05 were considered as statistically significant) in each TNFi at different time points during the study: visit 0 (prior starting TNFi), visit 1(pre-tapering), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1) and visit 4 (last visit available after visit 1).

Table II. Comparison of flares between tapering and control groups. The proportion of RA patients with flares, number of flares between visit 1-visit 4 and the time to appear the first flare in each TNFi are shown.

	Infliximab			Adalimumab			Etanercept		
RA* patient n = 144 patients	TG* n = 23	CG* n = 22	*p*	TG n = 23	CG n = 27	*p*	TG n = 21	CG n = 28	*p*
				Flares (n = 64 patients)					
Number of patients with flares, n/N(%)	11/23 (48%)	10/22 (46%)	0.9	9/23 (39%)	9/27 (33%)	0.7	6/21 (29%)	11/28 (39%)	0.4
Number of flares, mean ± SD*	2.2±0.8	2±0.7	0.26	1.6±0.7	1.9±0.7	0.5	1.3±0.5	1.5±0.7	0.8
Time to appear the 1st flare, mean ± SD (years)	1.1±0.8	1.5±0.7	0.07	1.6±1	0.8±0.4	0.1	1.4±0.5	1.7±0.7	0.5

*RA: rheumatoid arthritis; TG: tapering group; CG: control group; SD: standard desviation.
The number of patients with flares were compared using the Pearson χ^2 and Fisher exact tests. The number of flares and the time to appear the first flare were compared between groups using the Mann-Whitney U non-parametric test (p-values <0.05 were considered as statistically significant).



	Infliximab	Adalimumab	Etanercept
Visit 1	2112, 192-4160	3584, 1048-4979	1427, 835-2077
Visit 2	1280, 48-2560	1664, 422-2602	979, 324-1512
Visit 3	768, 20-2112	278, 26-1129	666, 493-933
Visit 4	160, 0-752	476, 12-756	200, 135-380

Ifx=infliximab, Ada=adalimumab, Etn=etanercept

Fig. 3. Serum trough drug levels along the study in RA patients belonging to the tapering group. The drug levels (Mdn, IQR ng/ml) of the different TNFi were measured during the study at different time points in the tapering group: visit 1 (pre-tapering), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1) and visit 4 (the last visit available after visit 1).

divided by TNFi. Most patients, after having a flare, reached the LDA at the end of the study (34 patients, 61%). The dropout in patients with flares was not statistically different between both groups [7/26 (27%) patients in the TG (6 patients due to inefficacy and 1 patient for other reasons) *vs.* 3/30 (10%) patients in the CG (all of them due to other reasons, p=0.099]. However, when we only consider the secondary inefficacy in patients with flare, it was observed that the dropout is more frequent in the TG (6/26 in TG *vs.* 0/30 in CG, p=0.005).

Twelve out of 26 patients (46%) with flare in TG needed to intensify the biological regimen after flaring and the 50% (6 patients) of them reached the control of the disease activity at the end of the study after therapy regimen intensification. In most patients with flares in both groups, temporary DMARDs intensifications, corticosteroids or NSAIDs were used to control the relapse.

The incidence of ADA appearance at the end of the study

Only 5 patients (in the TG: 1 patient with infliximab and 2 patients with adalimumab; in the CG: 2 patients with adalimumab) were ADA positive at pre-tapering (visit 1). Nineteen out of 136 patients (14%) had detectable ADA at the end of the study, and the majority of these patients were in the TG [13/65 (20%) ADA positive patients in the TG (7 with infliximab and 6 with adalimumab) *vs.* 6/71 (9%) ADA positive patients in the CG (4 with infliximab and 2 with adalimumab), p=0.052]. No ADA positive patients could be detected in the group of RA patients treated with etanercept. ADA was detected in 15 out of the 53 patients (28%) with a flare (11 patients in TG: 6 with infliximab and 5 with adalimumab; 4 patients in the CG: 3 with infliximab and 1 with adalimumab). Only two ADA positive patients needed to drop-out the therapy due to secondary inefficacy (1 patient with adalimumab in the TG and 1 patient with infliximab in the CG). At the end of the study, no differences were observed in clinical activity (DAS28) in patients who developed or not ADA at visit-4 in both groups (2.6±1 in ADA negative *vs.* 2.9±0.9 in ADA positive, p=0.159).

The influence of the tapering on serum drug levels in the tapering group (TG) during the study

A significant reduction in the drug levels was observed between visit 1 (pre-tapering) and visit 4 (at the end of the study) in the TG (data are shown in Fig. 3). Only two patients on adalimumab and 7 patients on etanercept had not available the drug levels at visit 1.

Predictors of a good clinical outcome to tapering strategy in the tapering group (TG)

In the tapering group, several demo-

graphic, clinical and serological factors were studied at baseline and at pre-tapering to predict what patients are more likely to present a flare during the tapering strategy (see Table III). The time in low disease activity previous to start the tapering strategy (OR: 0.35; 95% IC: 0.13□0.90) was the only predictive factor that demonstrated to be protector for having a flare (data shown in Table III).

Reduction of the administered drug during the study

At the end of the study (visit 4), RA patients in the TG received a significantly lower quantity of the administered drug in comparison with the standard therapy regimen (interval of administration in infliximab was 11.9±2.7 weeks, in adalimumab was 4.3±1.6 weeks and in etanercept was 2.1±0.9 weeks), with an interval elongation of approximately 33% in infliximab, 53% in adalimumab and 53% in etanercept. At the end of the study (visit 4), most patients in the TG were using the therapy regimen according to the tapering strategy without requiring the use of the standard labelled dose [19/23 (83%) in infliximab; 23/23 (100%) in adalimumab; 18/21 (86%) in etanercept].

Table III. Predictive power of clinical baseline and pre-tapering factors for having a flare during tapering strategy. Demographic, clinical and serological characteristics were analysed to predict a flare in rheumatoid arthritis patients under tapering strategy by means of univariate logistic regression analysis at baseline and pre-tapering.

Clinical factor	Odds ratio	95% CI
	At baseline	
Gender	3.87	0.78-19.3
RF*	0.74	0.22-2.47
ACPA*	1.21	0.41-3.58
Age	1.00	0.97-1.04
Disease duration	1.01	0.94-1.08
Smoking habit	1.07	0.23-4.89
Monotherapy use	1.68	0.38-7.41
	At pre-tapering	
Time in biological therapy	1.15	0.95-1.40
Time in inactive disease	0.35	0.13-0.9
DAS28*	1.96	0.85-4.52

*RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibodies; DAS28: disease activity score.

Discussion

To our knowledge, this is the first retrospective observational study that compares the clinical outcomes, drug levels and ADA appearance between RA patients at least in low disease activity treated with TNFi under a tapering strategy *versus* RA patients on the standard dosing regimen for a long-term follow-up. At the end of the study, most of the TG patients remained in low disease activity despite of the reduction in the amount of the administered drug in the TG. Another important issue to highlight is that there was no significant increase in the proportion of patients with a flare in the TG.

Several studies evaluated the withdrawal and down-titration strategy of TNFi in RA patients (15-28). In early RA patients, the discontinuation strategy in patients with sustained remission seems to be viable. However, in daily clinical practice, most RA patients on TNFi have longstanding disease, and for these subtypes of patients, evidence has shown that dose titration strategies are superior to discontinuation (15, 16, 21-28). Regarding the dose titration, three previous studies conducted in patients with long-standing RA demonstrated that this strategy is feasible in most patients without relevant changes in clinical outcomes (15, 16, 28). Additionally, the PRESERVE trial showed that RA patients on conventional or reduced doses of etanercept are more likely to maintain the low disease activity than patients who discontinued the therapy (26). Currently, there are no guidelines regarding tapering strategies in patients with sustained inactive disease. Our results have shown that tapering of TNFi results in a significantly lower amount of drug administered without a relevant increase in disease activity, leading to lower costs and preventing the development of long-term adverse effects, although the latter should be studied further (13).

One of the major concerns of a tapering strategy is the appearance of flares and dropout due to secondary inefficacy. In the HONOR study, it was found that the re-administration of adalimumab to patients with a flare was effective in re-achieving low disease activity within 6 months in most patients (22). Similar findings were observed in other studies after decreasing the dose in RA patients with stable disease (15, 16, 28). However, Saleem *et al.* showed that DAS28 remission rates after flaring were lower in RA patients who discontinued the TNFi (21). Previously, den Broeder *et al.* described that no RA patients under dose titration dropped out because of a persistent increase in the disease activity (15). In our cohort, 39% of RA patients developed flares during the follow-up without significant differences between the TG and CG. Another important issue is that most RA patients had low disease activity at the end of the study after experiencing a flare. Another concern about flares in clinical practice is whether the therapy intensification can resolve the flare without developing a therapy failure. We saw that most TG patients with flares after therapy intensification were in low disease activity at the end of the study. In general, the number of patients who dropped out after flaring was low in both groups, but this proportion was slightly higher in patients under TG. Additionally, when only it is considered the secondary inefficacy as reason to drop out, we could observe that the proportion of patients was very low (only 6 out of 144 (9%) patients) but all of them were in the TG being significant the differences between TG and CG. These findings reflect that though the frequency of undesirable outcomes in RA patients is low, it would be advisable to require a tight control of the disease activity in patients undergoing the tapering strategy.

In the TG, a progressive decrease in the drug levels was seen after starting the tapering strategy, and it has been established that ADA detection is more frequent in patients with low drug levels (33, 36, 40). This outcome suggests that patients with inactive disease probably need less amount of drug to keep disease activity under control and maybe drug and ADA measurements could be used as additional tools to monitor the disease activity in RA patients under a tapering strategy. Although, only 14% of our patients were ADA positive at the end of the study, it was seen that this proportion was slightly higher in the TG. However, very few ADA positive patients discontinued the therapy due to inefficacy (1 patient in the TG and 1 patient in the control group). Also, it is important to remark that the control of the disease activity was similar between ADA positive and ADA negative patients at the end of the study. This finding could be due to the drug is not the main factor influencing on the control of the clinical activity in ADA positive patients with low disease activity.

RA patients show a large variability in their response to TNFi, and the prediction of response remains an essential challenge (41-43). No robust protein biomarkers have been confirmed as predictors of response to a TNFi (41-43). The response prediction to the drug seems to be a multi-factorial event requiring multidisciplinary research (41-43). There is an increasing need to determine an individualised therapy strategy in RA patients based on strong predictors of response (42, 43). For now, the heterogeneity in the design of studies makes it difficult to draw robust conclusions (41-43). In the current study, several baseline and pre-tapering characteristics were analysed in order to find predictive values of having a flare after tapering. Our results showed that only a shorter time in inactive disease before the tapering strategy was predictive of flares after tapering, although patient numbers may not have been large enough.

Although different studies have determined that the medical costs of RA have increased since the introduction of TNFi (8-10, 12), significant savings in other sectors of healthcare have also been reported (11). Additionally, biological therapy could result in indirect cost savings as a consequence of a reduction in productivity losses and improving workforce participation (12, 44). Van der Maas *et al*. observed an important cost reduction in a cohort of RA patients after infliximab dose tapering (16). Another Spanish study in RA patients receiving TNFi, including patients with labelled, reduced or escalated dose regimens, found a cost reduction in patients treated with adalimumab or etanercept but not with infliximab (45). In our study, it was not possible to calculate the precise cost savings due to the differences in tapering strategies, however, the reductions in the administered drugs were significant, resulting in less direct costs (costs of the biologic agent) without an increase in disease activity.

We are conscious that this study has some limitations as patients are from different countries, the retrospective design and the small number of patients. As we explained previously, although included patients are from different countries, both cohorts were matched before to be included to ensure both were as homogenous as possible. Due to the strict criteria in the selection period many patients were excluded. Although the design is retrospective, these data reflect the type of patients that we usually find in daily clinical practice. Another limitation is that data about radiographic progression and disability are not available. In the present study, we use patients from clinical practice and not all patients have measured these parameters at the same time, making very difficult to obtain firm conclusions about this topic. Nevertheless, it would be interesting to take into account this issue in further studies to compare if there are differences in the radiographic evolution and even in the functional capacity between patients under a tapering strategy in comparison with patients under standard TNFi regimen.

In conclusion, the tapering strategy in RA patients with low disease activity seems to be feasible, resulting in a significant reduction in the amount of administered drug, while the disease control remains similar to RA treated via a standard dosing regimen. However, RA patients receiving tapering were more prone to discontinue the drug due to secondary inefficacy and ADA development.

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ARTICLE 11

TITLE: ***"Comparing Tapering Strategy to Standard Dosing Regimen of Tumor Necrosis Factor Inhibitors in Patients with Spondyloarthritis in Low Disease Activity."***

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PATIENTS AND METHODS:

Patients, clinical assessment and therapy regimen

In this retrospective observational study, 2 SpA cohorts taking TNFi were analyzed: a cohort from Spain under a tapering strategy (tapering group: TG) and a cohort from the Netherlands taking a standard therapy regimen (control group: CG). First, 528 patients with SpA (282 from Spain and 246 from the Netherlands) under TNFi (Ifx, Ada and Etn) were recruited, but after the selection period only 117 patients with SpA fulfilled the inclusion criteria (74 patients from Spain and 43 patients from the Netherlands). The number of patients from the Netherlands was lower because no control group was available for Ifx and during the matching process of both cohorts; several patients with SpA were excluded. All the selected patients with SpA had axial involvement and 49% (58) of them had also had some peripheral manifestations (arthritis, enthesitis, and dactylitis). The patients with AS fulfilled the revised New York criteria, and the remaining patients with SpA with non-radiographic axial SpA fulfilled the Assessment of Spondyloarthritis Society classification and diagnostic criteria. All the included patients with SpA had a sustained LDA of at least 6 months, defined by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) < 4, and also fulfilled 1 of these conditions: normal C-reactive protein (CRP) or ΔBASDAI > 50%.

Disease activity was measured by BASDAI at the different time points: visit 0 (prior to starting TNFi), visit 1 (before starting the tapering strategy in TG and after at least 6 months with LDA in the TG and CG), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1), visit 4 (the last visit available after visit 1), and visit flare [visit with (the worst) flare between visit 1 and visit 4]. Clinical activity was monitored every 6 months during the study, and the same periods of clinical evaluations were considered in the control group to avoid overestimating flares.

The tapering strategy was done as follows: in Ifx-treated patients, the tapering strategy included a gradual dose reduction (5 mg/kg to 4 mg/kg to 3 mg/kg) and/or interval administration per weeks (8 weeks to 9 weeks to 10 weeks, to a maximum of 15 weeks), ADA administration was prolonged 1 week until a maximum of 6 weeks and ETN was delayed 3 days for a maximum of 3 weeks as long as the physician decided that the interval of administration could be modified based on clinical and serological markers. The CG continued the standard therapy regimen throughout the study. The patients gave written informed consent prior to the start of the biological therapy.

Flares were recorded during the follow-up after visit 1 and were defined as BASDAI ≥ 4 and a ΔBASDAI ≥ 2 in comparison with the BASDAI at pre-tapering (visit 1). In the TG, in a flare episode, the TNFi dose could be increased or the interval could be shortened to regain low disease activity. When a flare was registered in the CG, an intense regimen of non-steroidal anti-inflammatory drugs (NSAID) and/or non-biologic disease-modifying anti-rheumatic drugs (DMARD) was used to control the disease activity.

In the selection period, the first step was to select a SpA cohort from Spain under tapering strategy who fulfilled the inclusion criteria. Later, both cohorts were matched according to several demographic, serological, and clinical characteristics to ensure that both groups were similar at baseline and at visit 1 (before starting the tapering strategy).

Serum samples and assays to measure drug and antidrug antibody levels

The serum drug concentrations (Ifx, Ada, and Etn) were determined by ELISA. A radioimmunoassay was performed to detect antidrug antibodies in the patients with SpA.

Statistical analysis

First, descriptive analyses were performed for the demographic and clinical variables. The results are shown as means and SD for continuous variables and relative frequencies for categorical variables. The frequency data were compared using the Pearson chi-squared and Fisher exact tests. The continuous data were compared between groups using the Mann-Whitney U and Wilcoxon nonparametric tests. Later, the associations between the independent variables and the outcomes were investigated using a univariate logistic regression model. Estimates for these associations are shown as standardized linear coefficient. SPSS 20.0 software was used for the analyses, and p values < 0.05 were considered statistically significant.

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Comparing Tapering Strategy to Standard Dosing Regimen of Tumor Necrosis Factor Inhibitors in Patients with Spondyloarthritis in Low Disease Activity

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ABSTRACT. ***Objective.*** To compare clinical outcomes, incidence of flares, and administered drug reduction between patients with spondyloarthritis (SpA) under TNF inhibitor (TNFi) tapering strategy with patients receiving a standard regimen.

Methods. In this retrospective study, 74 patients with SpA from Spain on tapering strategy (tapering group; TG) were compared with 43 patients from the Netherlands receiving a standard regimen (control group; CG). The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was measured at visit 0 (prior to starting the TNFi), visit 1 (prior to starting tapering strategy in TG and at least 6 months with BASDAI < 4 after starting the TNFi in the TG and CG), visit 2 (6 mos after visit 1), visit 3 (1 year after visit 1), and visit 4 (the last visit available after visit 1).

Results. An overall reduction of the administered drug was seen at visit 4 in the TG [dose reduction of 22% for infliximab (IFX) and an interval elongation of 28.7% for IFX, 45.2% for adalimumab, and 51.5% for etanercept] without significant differences in the BASDAI between the groups at visit 4 (2.15 ± 1.55 in TG vs 2.11 ± 1.31 in CG, p = 0.883). The number of patients with flares was similar in both groups [22/74 (30%) in the TG vs 8/43 (19%) in the CG, p = 0.184].

Conclusion. The tapering strategy in SpA results in an important reduction of the drug administered, and the disease control remains similar to that of the patients with SpA receiving the standard regimen.

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Key Indexing Terms:
SPONDYLOARTHRITIS | TNF INHIBITORS | TREATMENT
CLINICAL OUTCOMES | TAPERING

There is a growing interest in optimizing biological therapy in patients with spondyloarthritis (SpA), because the costs of this treatment are high, the longterm risks are unknown, and the treatment options are limited[1,2,3,4,5,6,7,8,9]. The therapeutic strategy once low disease activity (LDA) is achieved has not been clearly identified and little evidence is available regarding the predictors of maintaining LDA after lowering TNFi dose in patients with SpA[10]. Considering that TNF

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inhibitors (TNFi) are the only biologicals available in patients with SpA, changes in the therapy regimen should be made with caution. In a study of patients with ankylosing spondylitis (AS) taking infliximab (IFX), a stable clinical course was observed despite decreased doses and extended intervals of administration during the 1-year study period[6]. Another study in patients with AS taking etanercept (ETN) showed that remission was maintained in a high percentage of patients after halving the dose[1].

Several studies have demonstrated an association between the serum drug levels and the clinical response[11,12,13,14]. In a study of patients with rheumatoid arthritis (RA) who were treated with adalimumab (ADA), the optimal drug levels for maintaining a good clinical course were defined[15]; nevertheless, the optimal drug levels required to maintain stability in LDA or remission are unknown. The effect of biological therapies depends on the concentration and the immunogenic properties of these drugs[16]. It is beneficial to consider the pharmacokinetics of TNFi in the care of patients with SpA to optimize treatment and to reduce the risk of under- or overtreatment.

The introduction of TNFi into the management of AS and psoriatic arthritis has increased treatment costs[17,18,19,20]. A number of economic evaluations have been performed. A comparison of different TNFi found less favorable cost-effectiveness results for IFX[17,18,20]; however, these findings should be interpreted cautiously because of the variability in the dose regimen and drug pricing. Actual clinical data on TNFi for longterm use have not been published. The use of the tapering strategy in SpA patients with LDA might lead to cost reductions.

In recent years there has been a tendency at La Paz University Hospital, Madrid, Spain, to use a tapering strategy, with drug and anti-drug antibody level monitoring in patients with SpA who have sustained low disease activity. Conversely, in the Netherlands, the label dose is maintained even when a good clinical response has been registered in patients with SpA. Our main objectives were to compare the longterm clinical disease activity, incidence of flares, and incidence of antidrug antibodies at the end of the study between patients with SpA under a tapering strategy versus patients with SpA taking a standard dose. Our secondary targets were analyzed only in the SpA tapering group: the change in serum drug levels (IFX, ADA, or ETN) during the study, and predictors associated with good response to tapering.

MATERIALS AND METHODS

Patients, clinical assessment, and therapy regimen. In this retrospective observational study, 2 SpA cohorts taking TNFi were analyzed: a cohort from Spain under a tapering strategy (tapering group: TG) and a cohort from the Netherlands taking a standard therapy regimen (control group: CG). First, 528 patients with SpA (282 from Spain and 246 from the Netherlands) under TNFi (IFX, ADA, and ETN) were recruited, but after the selection period only 117 patients with SpA fulfilled the inclusion criteria (74 patients from Spain and 43 patients from the Netherlands). The number of patients from the Netherlands was lower because no control group was available for IFX and during the matching process of both cohorts, several patients with SpA were excluded (Appendix 1).

All the selected patients with SpA (87 patients with AS, 11 patients with nonradiographic SpA, 8 with SpA associated with inflammatory bowel disease, and 11 psoriatic patients) had axial involvement and 49% (58) of them had also had some peripheral manifestations (arthritis, enthesitis, dactylitis). The patients with AS fulfilled the revised New York criteria, and the remaining patients with SpA with nonradiographic axial SpA fulfilled the Assessment of Spondyloarthritis Society classification and diagnostic criteria[21,22]. All the included patients with SpA had a sustained LDA of at least 6 months, defined by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) < 4, and also fulfilled 1 of these conditions: normal C-reactive protein (CRP) or ΔBASDAI > 50%.

Disease activity was measured by BASDAI at the different timepoints: visit 0 (prior to starting TNFi), visit 1 (before starting the tapering strategy in TG and after at least 6 mos with LDA in the TG and CG), visit 2 (6 mos after visit 1), visit 3 (1 year after visit 1), visit 4 (the last visit available after visit 1), and visit flare [visit with (the worst) flare between visit 1 and visit 4]. Clinical activity was monitored every 6 months during the study, and the same periods of clinical evaluations were considered in the control group to avoid overestimating flares.

The tapering strategy was done as follows: in IFX-treated patients, the tapering strategy included a gradual dose reduction (5 mg/kg to 4 mg/kg to 3 mg/kg) and/or interval administration per weeks (8 weeks to 9 weeks to 10 weeks, to a maximum of 15 weeks). ADA administration was prolonged 1 week until a maximum of 6 weeks and ETN was delayed 3 days for a maximum of 3 weeks as long as the physician decided that the interval of administration could be modified based on clinical and serological markers. The CG continued the standard therapy regimen throughout the study. The patients gave written informed consent prior to the start of the biological therapy for the use of their clinical data and serum for research.

Flares were recorded during the followup after visit 1 and were defined as BASDAI ≥ 4 and a ΔBASDAI ≥ 2 in comparison with the BASDAI at pre-tapering (visit 1). In the TG, in a flare episode, the TNFi dose could be increased or the interval could be shortened to regain low disease activity. When a flare was registered in the CG, an intense regimen of nonsteroidal antiinflammatory drugs (NSAID) and/or nonbiologic disease-modifying antirheumatic drugs (DMARD) was used to control the disease activity.

In the selection period, the first step was to select an SpA cohort from Spain under tapering strategy who fulfilled the inclusion criteria. Later, both cohorts were matched according to several demographic, serological, and clinical characteristics to ensure that both groups were similar [age, sex, disease duration, HLA-B27 positivity, the disease activity (BASDAI) at baseline and at visit 1 (before starting the tapering strategy), duration of inactive disease prior to visit 1, and the time of followup between visit 1 and visit 4]. All included patients were white. Patients with SpA who did not fulfill these requirements were excluded from the study to avoid misinterpretations using heterogeneous cohorts (Appendix 1).

Serum samples and assays to measure drug and antidrug antibody levels. Blood samples were collected a maximum of 24 h before drug administration for subcutaneous TNFi or immediately before intravenous infusions of IFX. The serum drug concentrations (IFX, ADA, and ETN) were determined by ELISA, as described previously[13,23,24]. A radioimmunoassay was performed to detect antidrug antibodies in the patients with SpA, as previously described[12,23,25].

Statistical analysis. First, descriptive analyses were performed for the demographic and clinical variables. The results are shown as means and SD for continuous variables and relative frequencies for categorical variables. The frequency data were compared using the Pearson chi-squared and Fisher exact tests. The continuous data were compared between groups using the Mann-Whitney U and Wilcoxon nonparametric tests. Later, the associations between the independent variables and the outcomes were investigated using a univariate logistic regression model. Estimates for these associations are shown as standardized linear coefficient. SPSS 20.0 software was used for the analyses, and p values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics. In Table 1 the demographic characteristics are shown comparing the TG versus CG. Most patients were receiving monotherapy in the CG. The time in LDA prior to visit 1 was higher in the TG although not statistically different. Patients taking IFX had more time in LDA prior to the tapering strategy, but in patients taking ETN and ADA, this time was very similar (IFX: 1.5 ± 1.3 yrs in TG; ADA: 0.7 ± 0.2 yrs in TG vs 0.8 ± 0.5 yrs in CG, p = 0.6; ETN: 0.8 ± 0.1 yrs in TG vs 0.9 ± 0.4 yrs in CG, p = 0.5).

Clinical response during the study. The clinical course measured by BASDAI was similar in the 2 groups during the study (Figure 1). In a subgroup analysis to compare the clinical activity between the various TNFi, no significant differences were observed (Figure 2). The patients in the TG taking ETN had higher clinical activity at visit 2; however, this difference was not significant (Figure 2).

The majority of patients with SpA had LDA at the end of the study [63/74 (85.1%) in the TG vs 39/43 (90.7%) in the CG at visit 4, p = 0.386], even after a subanalysis comparing the 2 groups per TNFi [IFX: 30/35 (85.1%) in the TG at visit 4; ADA: 15/17 (88.2%) in the TG vs 19/21 (90.5%) in the CG at visit 4, p = 0.823; ETN: 18/22 (81.8%) in the TG vs 20/22 (90.9%) in the CG at visit 4, p = 0.380].

Flares during the study. Thirty patients with SpA (26%) experienced a flare during our study [22/74 (30%) in the TG vs 8/43 (19%) in the CG, p = 0.184]. No differences were observed in the number of flares between groups (1.4 ± 0.7 in the TG vs 1.5 ± 0.5 in the CG, p = 0.486) or in the time to the first flare after visit 1 (1.3 ± 0.8 yrs in the TG vs 1.3 ± 1.2 yrs in the CG, p = 0.841). Table 2 shows the proportion of patients with flares, the number of flares, and the time to the first flare for patients of the TG and CG divided by TNFi. Most patients, after having a flare, reached the LDA at the end of the study (19 patients, 63%). Three out of 22 patients in the TG dropped out of the therapy because of inefficacy, and no patients in the CG with flare needed to discontinue the therapy (only 1 patient discontinued in the CG, because of an adverse event).

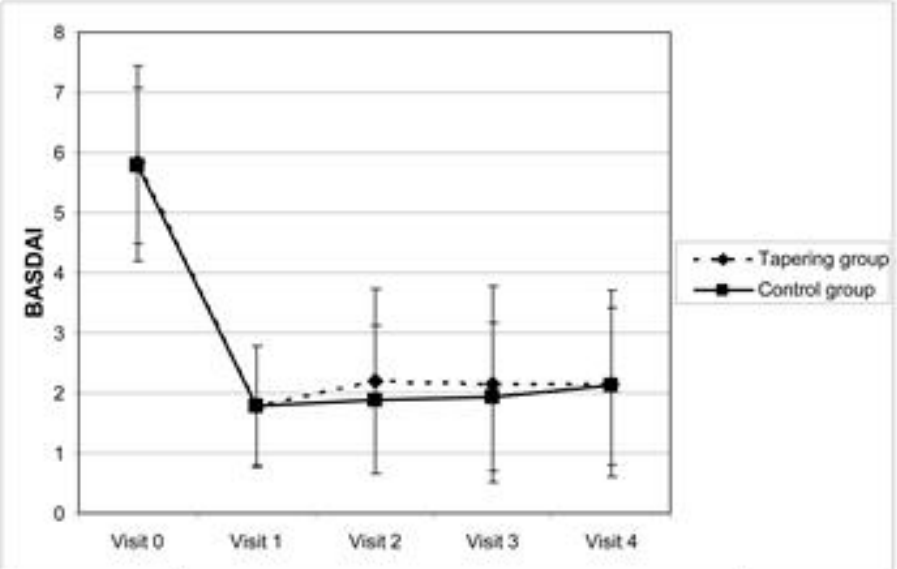
In the 22 patients under tapering strategy, more patients with flare were in the IFX group [IFX: 14/35 (40%); ADA: 2/17 (12%); ETN: 6/22 (27%); p = 0.108]. In the TG, the nonbiologic DMARD were intensified in 1 patient and the NSAID were used at flare in 13 patients. Most patients in the TG with a flare who were treated with IFX or ADA needed to increase the dose or shorten the interval of administration to regain control over the disease activity [IFX: 13/14 (93%); ADA: 2/2 (100%); ETN: 2/6 (33.3%)]. The clinical activity at the worst registered flare in the TG was lower in the ETN patients (IFX: 5.9 ± 1.2; ADA: 6.3 ± 0.4; ETN: 4.7 ± 0.5; p = 0.028). In the CG, 7 patients with flares intensified NSAID and 1 patient started nonbiologic DMARD.

The incidence of antidrug antibody appearance at the end of our study. Only 2 patients treated with IFX in the TG were positive for antidrug antibodies at pre-tapering (visit 1). Sixteen patients (14%) had detectable antidrug antibodies at

Table 1. Demographic characteristics of 117 patients with SpA.

SpA Patients, n = 117	TG, n = 74	CG, n = 43	p
Male, n (%)	54 (73)	31 (72)	0.9
Age, yrs, mean ± SD	50.3 ± 12.5	47 ± 9.3	0.2
Disease duration, yrs, mean ± SD	15.2 ± 9.3	14.4 ± 7.6	0.9
HLA-B27, n (%)	54/59 (91)	41/43 (95)	0.45
Baseline BASDAI, mean ± SD	5.8 ± 1.6	5.8 ± 1.3	0.96
Baseline CRP, mg/l, mean ± SD	14.4 ± 23.7	15 ± 15.7	0.2
Subtypes of SpA, n (%)			0.183
Ankylosing spondylitis	51 (70)	36 (84)	
Nonradiographic SpA	8 (10)	3 (7)	
SpA associated to inflammatory bowel disease	5 (7)	3 (7)	
Psoriatic SpA	10 (13)	1 (2)	
Prior biological use, n (%)	10 (14)	5 (12)	0.7
Duration of low disease activity prior to visit 1, yrs, mean ± SD	1.2 ± 1.1	0.7 ± 0.2	0.24
Duration of followup between visit 1 and visit 4, yrs, m ± SD	2.3 ± 1.1	2.4 ± 1	0.6
Baseline co-therapy, n (%)			
Methotrexate only (MTX)	11 (15)	3 (7)	0.2
Other DMARD only (OD)	17 (23)	6 (14)	0.2
MTX + OD	8 (11)	1 (2)	0.1
TNFi monotherapy	38 (51)	33 (77)	0.007

SpA: spondyloarthritis; TG: tapering group; CG: control group; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drugs; TNFi: tumor necrosis factor inhibitor.



	BASDAI		p
	Tapering Group	Control Group	
Visit 0	5.8 ± 1.6	5.8 ± 1.3	0.96
Visit 1	1.8 ± 1	1.8 ± 1	0.7
Visit 2	2.2 ± 1.5	1.9 ± 1.2	0.5
Visit 3	2.2 ± 1.6	1.9 ± 1.2	0.7
Visit 4	2.2 ± 1.5	2.1 ± 1.3	0.9

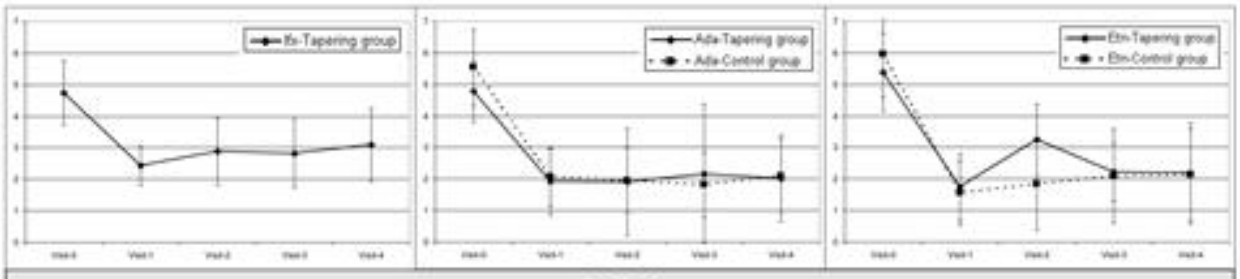
Figure 1. Comparison of the clinical activity (BASDAI) between tapering and control groups. The clinical evolution was measured by BASDAI (mean ± SD) at different timepoints during the study: visit 0 (prior starting to starting TNFi), visit 1 (pre-tapering), visit 2 (6 mos after visit 1), visit 3 (1 yr after visit 1), and visit 4 (last visit available after visit 1). BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; TNFi: tumor necrosis factor inhibitors.

the end of our study, and the majority of these patients were in the TG [14/73 (19.2%) antidrug antibody–positive in the TG (11 with IFX and 3 with ADA) vs 2/43 (4.7%) in the CG (all with IFX), p = 0.028]. No antidrug antibody–positive patients could be detected in the group of patients with SpA treated with ETN. Antidrug antibodies were detected in 6 out of the 30 patients (20%) with a flare (5 patients taking IFX in TG and 1 patient taking ADA in CG), but only 2 patients under IFX in the TG needed to drop the therapy because of secondary inefficacy. At the end of our study, no differences were observed in clinical activity (BASDAI) in patients who developed or not antidrug antibodies at visit 4 in both groups (TG: 2.2 ± 1.6 in antidrug antibody–negative vs 2.0 ± 1.6 in antidrug antibody–positive, p = 0.659; CG: 2.1 ± 1.3 in antidrug antibody–negative vs 2.3 ± 0.2 in antidrug antibody–positive, p = 0.603).

The influence of the tapering on serum drug levels. A significant reduction in the drug levels was observed between visit 1 (pre-tapering) and visit 4 (at the end of our study) in the TG (Figure 3). Only 2 patients taking ADA and 7 patients taking ETN did not have the drug levels available at visit 1.

Predictors of a good clinical outcome to tapering strategy. In the tapering group, several demographic, clinical, and serological factors were studied at baseline and at pre-tapering to predict which patients were more likely to present a flare during the tapering strategy (Table 3). Being male (OR 3.5; 95% CI 1.18-10.4) was the only predictive factor that demonstrated to be protective for having a flare (Table 3).

Reduction of the administered drug in the tapering group during the study. At the end of the study (visit 4), the patients with SpA in the TG received a substantially lower amount of drug compared with the patients in the CG (IFX dose was 4.40 ± 0.81 mg/kg; interval of administration for IFX was 11.22 ± 1.80 weeks; for ADA, 3.74 ± 1.21 weeks, and for ETN, 2.09 ± 0.59 weeks).



BASDAI							
	IFX	ADA			ETN		
	TG	TG	CG	p	TG	CG	p
Visit 0	6.3 ± 1.72	4.8 ± 1	5.5 ± 1.2	0.07	5.4 ± 1.2	6 ± 1.4	0.3
Visit 1	1.7 ± 1	1.9 ± 1	2.1 ± 0.9	0.5	1.7 ± 1.1	1.5 ± 1	0.6
Visit 2	2.1 ± 1.9	1.9 ± 1.7	1.9 ± 1	0.6	3.2 ± 1.1	1.8 ± 1.5	0.090
Visit 3	2.1 ± 1.6	2.2 ± 2.2	1.8 ± 1	0.9	2.2 ± 0.9	2.1 ± 1.5	0.5
Visit 4	2.2 ± 1.6	2 ± 1.4	2.1 ± 1.2	0.8	2.2 ± 1.6	2.1 ± 1.5	0.9

Figure 2. Comparison of clinical activity (BASDAI) between tapering and control groups in each TNFi. The clinical activity was measured by BASDAI (mean ± SD, represented in X-axes) in each TNFi at different timepoints during the study: visit 0 (prior starting TNFi), visit 1 (pre-tapering), visit 2 (6 mos after visit 1), visit 3 (1 yr after visit 1), and visit 4 (last visit available after visit 1). TG: tapering group; CG: control group; IFX: infliximab; ADA: adalimumab; ETN: etanercept; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; TNFi: tumor necrosis factor inhibitors.

Table 2. Comparison of flares between tapering and control groups. The proportion of SpA patients with flares, number of flares between visit 1 and visit 4, and the time to first flare in each TNFi are shown.

SpA patients, n = 117	IFX TG, n = 35	CG, n = 21	ADA TG, n = 17	p	CG, n = 22	ETN TG, n = 22	p
Flares, n = 30 patients							
No. patients with flares, n/N (%)	14/35 (40)	3/21 (14)	2/17 (12)	0.431	5/22 (23)	6/22 (27)	0.498
No. flares, mean ± SD	1.5 ± 0.7	1.4 ± 0.6	2 ± 1.4	0.519	1.2 ± 0.5	1.6 ± 0.4	0.615
Time to appearance of first flare, yrs, mean ± SD	1.2 ± 0.5	0.9 ± 0.6	1 ± 0.1	1.000	1.6 ± 1.4	1.9 ± 1.5	0.156

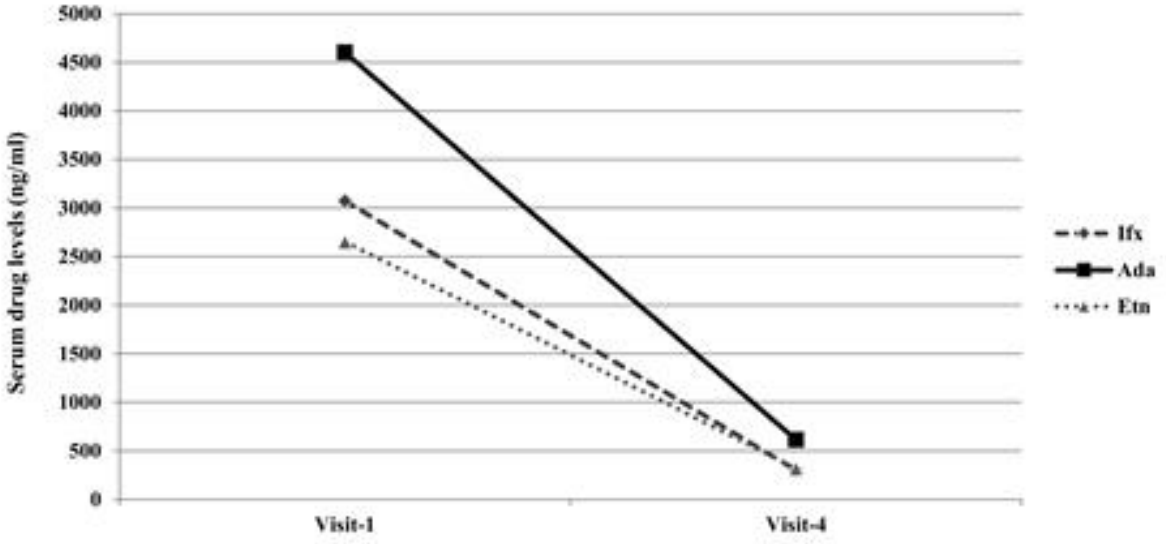
SpA: spondyloarthritis; TG: tapering group; CG: control group; IFX: infliximab; ADA: adalimumab; ETN: etanercept.

Overall, the reduction of the administered drug at visit 4 in the TG was 22% for IFX, and the interval was extended to 28.7%. The dose reduction was 45.2% for ADA and 51.5% for ETN. The majority of the patients in the tapering group continued with the tapering strategy at visit 4 [34/35 (97.1%) taking IFX; 16/17 (94.1%) taking ADA; 19/22 (86.4%) taking ETN].

DISCUSSION

To our knowledge, this work is the first retrospective observational longterm followup study comparing the clinical and serological outcomes between patients with SpA using a tapering strategy versus a standard regimen in daily clinical practice. Although an important reduction in the administered drug was achieved in the TG (IFX dose 22% and interval 28.7%; ADA interval 45.2%; ETN interval 51.5%), the percentage of patients who maintained a BASDAI < 4 at the end of our study was similar in both groups. The development of flares was low during the study but the frequency was a little higher in patients under the tapering strategy, mainly in patients receiving IFX.

The evidence regarding the discontinuation and dose titration of TNFi in patients with SpA is sparse and varied[1,2,4,5,6]. Most of the studies that focused on TNFi discontinuation failed to demonstrate that this strategy resulted in good control of disease activity. These studies included heterogeneous populations, different outcome measurements, and variable followup periods, making it difficult to extrapolate the results to other patient populations[4,5,8]. The evidence for dose-titration in patients with SpA is inconclusive[1,6]. In patients with AS who were treated with ETN, Cantini, *et al* observed that remission was possible in at least 50% of the patients and remission was maintained in the majority of patients after halving the dose[1]. Similar results were seen in patients with AS treated with IFX in cases in which the clinical improvement was sustained during the



	Infliximab (Mdn, IQR)		Adalimumab (Mdn, IQR)		Etanercept (Mdn, IQR)	
Visit 1	n = 35	3072 (1792–4992)	n = 15	4600 (2146–4600)	n = 15	2652 (1091–2662)
Visit 4	n = 35	300 (0–1664)	n = 17	614 (267–1241)	n = 22	318 (133–409)
p	< 0.001		0.001		0.001	

Figure 3. The decrease of serum trough drug levels in patients with SpA under tapering strategy during the study. The drug levels (Mdn, IQR ng/ml) of the different TNFi were measured during the study at different timepoints in the tapering group: visit 1 (pre-tapering) and visit 4 (the last visit available after visit 1). Not all patients had the serum drug levels at visit 1 in ADA and ETN. SpA: spondyloarthritis; Mdn: median; IQR: interquartile range; IFX: infliximab; ADA: adalimumab; ETN: etanercept; TNFi: tumor necrosis factor inhibitors.

Table 3. Predictive clinical baseline and pre-tapering factors predicting a flare during tapering strategy. Demographic, clinical, and serological characteristics were analyzed to predict a flare in patients with SpA under tapering strategy by means of univariate logistic regression analysis at baseline and pre-tapering.

Predictive Factor	OR	95% CI
At baseline		
Male sex	3.50	1.18–10.40
Age	1.03	0.99–1.07
Disease duration	0.98	0.92–1.04
Naive to biologicals	0.99	0.23–4.26
HLA-B27	0.31	0.05–2.1
Monotherapy	0.55	0.20–1.51
At pre-tapering		
Time in inactive disease	1.22	0.75–1.98
BASDAI	1.51	0.91–2.52
CRP levels	1.03	0.89–1.20

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; SpA: spondyloarthritis.

course of the study despite a reduced dose and longer infusion intervals[6]. In our study, we showed that the clinical course in the TG was similar to that in the CG. These findings suggest that the tapering strategy is superior to discontinuation of the TNFi in patients with SpA who have LDA.

In considering a tapering strategy for patients with SpA with sustained LDA, one of the most important concerns of rheumatologists is the increased risk of flares and the inefficacy of TNFi after a flare. However, most publications regarding withdrawal of biological therapies in patients with SpA have shown that re-starting is safe and effective in most patients[1,5,8]. Our data demonstrate that 26% of the patients developed a flare during the study. The number of patients with a flare was slightly higher in the TG, without significant differences. An important issue is that more than 60% of these patients had inactive disease at the end of the study; the dropout rate due to inefficacy was very low. No patients taking ETN in our study dropped out after flaring; a probable explanation is that the median of clinical activity in flares was lower in these patients when comparing with IFX or ADA. The data about therapeutic changes on biological and classic DMARD after flaring were collected. However, it was not possible to obtain proper data on the use of NSAID during flares because of the retrospective design of our study. Globally, these data reflect that even in a selected SpA cohort in LDA, flares are present during the followup in patients

under tapering or standard therapy regimen, and tight clinical monitoring is needed to make therapeutic decisions as soon as possible to avoid undesirable outcomes.

In general, antidrug antibody detection was low in patients in our study (14%), but it should be noted that it was more frequent in patients on tapering strategy who were treated with IFX. It is widely known that antidrug antibody detection is more frequent in patients with low drug levels[23]. These results should be studied in a larger population to investigate whether dose tapering of TNFi results in more inefficacy (hence, more dropouts) because of antidrug antibody development. A study showed that patients with SpA who develop antidrug antibody–positivity to the first TNFi have a good clinical response after switching to a second TNFi[24]. However, patients who developed antidrug antibodies to the first TNFi were more prone to present with antidrug antibodies to the second TNFi[26]. Prior to our present report, there had been no evidence about what happens with drug levels and antidrug antibody appearance when a tapering strategy is carried out in patients with SpA in LDA. Our data show that patients under tapering strategy had a progressive decrease of drug levels after tapering and also presented more frequency of antidrug antibody, although the data are sparse. But these findings in our cohort are not associated with a higher incidence of flares or dropouts, indicating that, in some patients, the disease may be completely inactive and the drug may not be the main factor that influences this status. Currently, there are some doubts about whether drug and antidrug antibody measurements are useful in patients on a TNFi dose-tapering strategy.

Several studies of randomized clinical trials and registries have attempted to identify predictors of the responses to TNFi in patients with AS[27,28,29,30,31]. Data from registries have shown that elevated inflammatory markers, a lower Bath Ankylosing Spondylitis Functional Index, and younger age at baseline were associated with better clinical responses. In a prospective observational cohort study in patients with AS treated with TNFi, these factors were observed to be independent baseline predictors of responses and/or continuation of TNFi[32]: higher Ankylosing Spondylitis Disease Activity Score, higher ESR or CRP levels, the presence of peripheral arthritis, younger age, male sex, a lower modified Schöber test, and lower BASDAI. Predictive markers of having a flare after tapering strategy in patients with SpA have not been previously described. In our cohort, we found that male sex was a predictive factor to protect from flare when dose titration was made in patients with SpA in low disease activity.

Biological treatment is expensive; therefore, tapering strategies have important economic implications. In a study of patients with RA in which IFX was down-titrated or discontinued, a mean cost reduction of 3474 euros (US$3883) per patient was observed during 1 year[33]. From the results of our study, it is not possible to calculate the exact financial savings because of the differences in tapering strategies. However, an important reduction in the administered TNFi was reached, without relevant clinical changes, after tapering in patients with SpA. There were cost reductions, the patients were not overtreated, and they were less likely to develop potential adverse events or infections.

Our study had some limitations: the patients were from different countries, there was no control group for the patients treated with IFX, the design was retrospective, and the number of patients was small. Although included patients were from different countries, both cohorts were matched to ensure they were as homogeneous as possible. Because of the strict criteria in the selection period, many patients were excluded. One inconvenience of the study was not finding a control group for patients treated with IFX, but this drug is not used much in the patients with SpA from the Netherlands, and after matching the few Dutch patients, it was impossible to find a homogeneous group for comparison. On the other hand, it was very useful to show what happened to patients taking IFX when a tapering strategy was done, even if a control group was absent. Although the design was retrospective, these data reflect the type of patients that we usually find in daily clinical practice.

The tapering strategy in patients with SpA with low disease activity appears to be feasible, resulting in an important reduction of the administered drug; disease control remains similar to that of patients with SpA on the standard dosing regimen. The incidence of flares and antidrug antibody detection was low in both cohorts during our study, but a little higher in patients under the tapering strategy, indicating that a tight clinical and serological monitoring should be done in these patients to avoid unexpected clinical outcomes.

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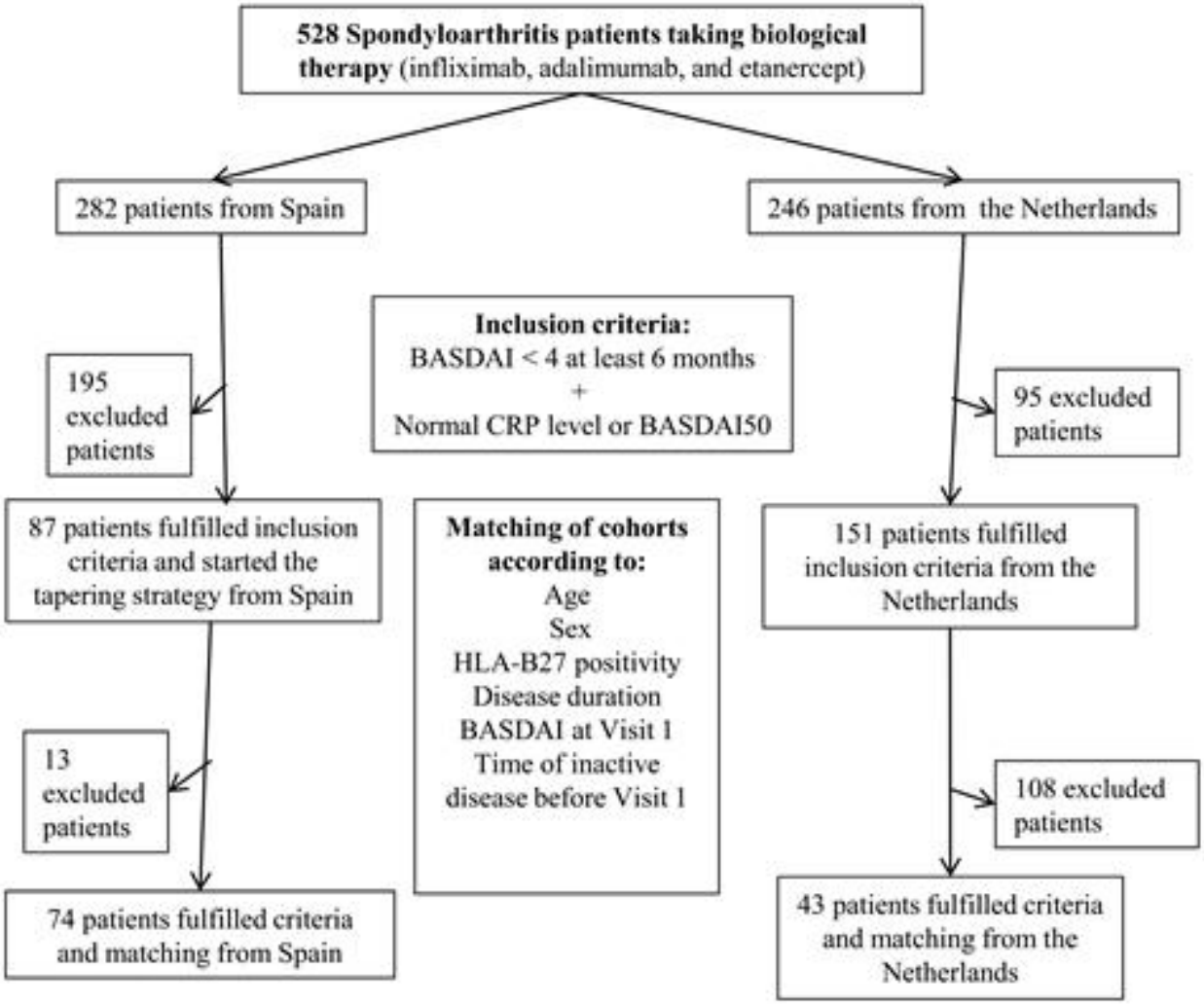
APPENDIX 1. Flowchart describing patient selection and inclusion. BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein.



Chapter 4: Economic repercussion of tapering strategies monitoring drug/ADA levels (TDM)

ARTICLE 12: ***"Dose-Tapering of TNF inhibitors in daily rheumatology practice enables the maintenance of clinical efficacy while improving cost-effectiveness"***

ARTICLE 13: ***"Anti-TNF dose and anti-drug antibody levels in rheumatic and psoriasis patients: economic repercussion"***

ARTICLE 12

TITLE: "*Dose-Tapering Of TNF Inhibitors in Daily Rheumatology Practice Enables the Maintenance of Clinical Efficacy While Improving Cost-Effectiveness*"

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PATIENTS AND METHDOS:

This is a retrospective observational study which develops in two different time periods. In the first period (1st P) from 2007 to 2009 (2.02 ± 0.84 years), the patients were treated with a standard therapy; in the second period (2nd P), from 2010 to 2012 (2.42 ± 0.33 years), they were treated with a tapering strategy. This study design allowed the patients to be their own controls because the same individuals were compared in both time periods.

To be included in the study, patients had fulfilled these criteria: i) to be for at least six months sustained LDA (defined in RA patients by the DAS28<3.2 and in axial SpA patients by the BASDAI<4 with one of these conditions: normal C-reactive protein (CRP) or delta-BASDAI>50%); ii) to be treated with the same TNF inhibitor throughout the entire study and iii) to have received treatment in both study periods.

Seventy seven patients (36 (46.7%) with RA and 41 (53.3%) with SpA) out of the total of 395 treated with Ifx, Ada or Etn in the Rheumatology Department from La

Paz University Hospital, met these inclusion criteria. All RA patients fulfilled the 2010 or 1987 ACR revised criteria and all SpA patients have axial involvement and fulfilled the New York revised criteria or the ASAS group criteria.

Clinical activity was evaluated at baseline and every 6 months by the DAS28 in RA patients and by the BASDAI in SpA patients. BASDAI was used to evaluate SpA patients instead of ASDAS because no ASDAS values were available at the beginning of the study period. This was an observational study that did not require the approval of the Hospital Ethical Committee.

The tapering strategy consisted in a progressive interval prolongation (increasing the interval of Ifx and Ada administration by one week and of Etn administration by 3 days) and/or dose reduction (decreasing by 1 mg/kg until 3 mg/kg in SpA patients treated with Ifx) following the physician criteria based on clinical and serological markers CRP, erythrocyte sedimentation rate-ESR- and TNFi levels). A flare was defined as an increase of the DAS28-ESR (a composite score measuring disease activity) greater than 3.2 plus a delta-DAS28 (related to pre-tapering DAS28) lower than -0.6 in RA patients and BASDAI $\geq$ 4 and delta-BASDAI $\leq$-2 (related to pre tapering BASDAI) in SpA patients in at least one clinical visit during the study. In the case of a flare, the concomitant therapy (disease-modifying anti-rheumatic drugs-DMARDS-, non-steroidal anti-inflammatory drug -NSAIDS-, corticosteroids) could be intensified in both periods, but the TNFi therapy was only increased in the 2nd P.

Out of the 77 patients, 29 were treated with Ifx, 27 with Ada, and 21 with Etn. In the 1st P, Ifx was administered intravenously to RA patients at 3 mg/kg at 0, 2, 6 weeks and every 8 weeks thereafter; and to patients with SpA at 5 mg/kg at 0, 2, 6 weeks and every 8 weeks thereafter. Ada was administered at 40 mg/2 weeks and Etn at 50 mg/ week following the drug labels. In the 2nd P, clinicians were allowed to increase the administration interval and/or reduce dose in case of Ifx if the patient was presenting LDA.

Blood samples were collected a maximum of 24h before the biologic drug administration for a subcutaneous TNF inhibitor or just before intravenous (iv) infusion for Ifx. Drug and ADA levels were measured at every visit for patients on Ifx treatment and every 6 months for patients on subcutaneous administration.

Measurement of drug and ADA concentration

Serum drug concentrations were determined by a ELISA. Cut-off values for positive drug levels were 10nag/ml for Ifx, 5nag/ml for Ada and 30nag/ml for Etn. Serum ADA levels were assayed using a two-site (bridging) in-house ELISA.

Evaluation of the dispensed amount of drug and cost analysis

The drug amount that each patient received was obtained from the database of the Pharmacology Department. We evaluated the mean dose delivered to the patient and the mean time elapsed between the dispensations in both periods of the study. With these data, the mean weekly dose was calculated. The cost of the treatment in each period was calculated taking the price of each medication at the end of 2012 into account.

Statistical analysis

Differences in baseline characteristics were assessed using Pearson's chi-square test and Fisher's exact test for ordinal variables. Continuous non-parametric data were compared between groups using the Mann- Whitney U test. Paired comparisons of parametric results were performed by Student's t-test and non-parametric results by the Wilcoxon matched-pairs test. p-values were considered significant when under 0.05. Statistics were performed using Graph Pad Prism6 software (San Diego, CA, EEUU).

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Research Open Access

Dose-Tapering Of TNF Inhibitors in Daily Rheumatology Practice Enables the Maintenance of Clinical Efficacy While Improving Cost-Effectiveness

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Abstract

Background

The fact that biologics consume a growing portion of health care budget has resulted in an increased attention towards therapy optimization. One of the potential ways to optimize treatment is the down-titration of the administered drug dose.

Objective

To assess whether the clinical activity remains stable after dose tapering of TNF inhibitors in patients with low disease activity and to evaluate the potential benefit of this strategy on the treatment costs.

Method

A cohort of 77 patients with low disease activity treated with TNF inhibitors (TNFi) was monitored. The patients were studied over two time periods: in the 1st period with the drug standard dose, and in the 2nd period with a reduced dose. Clinical efficacy was monitored by DAS28 in rheumatoid arthritis (RA) and by BASDAI in spondyloarthritis (SpA). Serum drug and anti-drug antibody levels were measured by ELISA. The amount of drug dispensed per patient in both periods was compared.

Results

In the 2nd period, although patients received a lower amount of TNF inhibitor, no differences in clinical activity were observed (DAS28 in RA patients: 2.37 ± 0.50 in the 2nd P vs 2.28 ± 0.47 in the 1st P, p=0.20; BASDAI in SpA patients: 1.90 ± 0.93 in the 2nd P vs 1.88 ± 0.95 in the 1st P, p=0.910) and circulating serum trough drug levels were lower (Infliximab: 3.2 ± 2.5 µg/ml in the 1st P vs 1.8 ± 1.5 µg/ml in the 2nd P, p<0.0001; Adalimumab: 5.5 ± 2.8 µg/ml in the 1st P vs 3.1 ± 2.1 µg/ml in the 2nd P, p<0.0001; Etanercept: 1.8 ± 1.1 µg/ml in the 1st P vs 1.3 ± 0.8 µg/ml in the 2nd P p<0.05). The amount of administered drug per patient was reduced in an average of 20% per year.

Conclusion

Dose tapering can be successfully performed in patients with low disease activity, resulting in remarkable savings in the amount of drug used and in the associated costs.

Keywords: Anti-TNFα therapy; Dose tapering; Cost-effectiveness; Clinical efficacy

Introduction

Biological drugs are far more costly than traditional treatments [1]. The fact that biologics consume a growing portion of health care budgets has resulted in an increased attention towards therapy optimization [2,3]. Recently, it has been shown that dose tapering of TNFi is a feasible therapeutic option in rheumatic patients with low disease activity (LDA) [4-10]. However, concerns about the risk of disease flares, the progression of radiological damage and the need to increase other medications with potential side effects have limited its implementation. In the last years some publications on tapering and discontinuation of TNFi have appeared [11-14], most of them taking part of randomized control trials. Others are based on disease activity guided strategy of TNFi dose reduction to discontinuation [15]. A high heterogeneity in patient's selection, study design and outcome

definition makes it difficult to draw conclusions, driving that active dose reduction strategies are not widely adopted by rheumatologists in clinical practice [5].

Several publications have demonstrated an association between the serum drug levels and the clinical response [16-19]. A good clinical response is mainly associated to elevated serum drug levels and a low frequency of anti-drug antibody detection. However, the optimal drug levels required to maintain stable LDA or clinical remission are unknown [20].

A careful follow-up of the clinical response to TNF inhibitor in combination with the monitoring of drug and antidrug antibody (ADA) levels, known as therapeutic drug monitoring (TDM) [17], can potentially influence prescribing procedures. TDM has been used in clinical practice to individualize the therapy of a small number of drugs, but it has been scarcely studied in the context of biological drugs. Some authors claim that the monitoring of drug and ADA levels can be extremely useful in guiding the dosage of these drugs [21-23]. However, other groups defend that only the clinical activity of patients under biological treatment is necessary to accurately control the amount of administered drug.

In the Biological Therapy Unit of the Rheumatology Department in our hospital, the determination of drug and ADA levels was introduced some years ago, but only in recent years have these parameters been taken into account as additional assessment tools for clinical monitoring. Using the accumulated experience since the year 2003 [18], our rheumatologists evaluate the disease activity together with the drug and ADA levels (TDM) to make decisions, such as switching treatments or dose de-escalation.

The main objective of the present work was to analyze whether RA and SpA patients that had attained at least LDA could maintain stable clinical activity while receiving lower dose than the standard treatment. The potential benefit on the treatment costs of such approach was also evaluated.

Patients and Method

This is a retrospective observational study which develops in two different time periods. In the first period (1st P) from 2007 to 2009 (2.02 ± 0.84 years), the patients were treated with a standard therapy; in the second period (2nd P), from 2010 to 2012 (2.42 ± 0.33 years), they were treated with a tapering strategy. This study design allowed the patients to be their own controls because the same individuals were compared in both time periods, maintaining homogeneity in body mass index, concomitant diseases and genetic background.

To be included in the study, patients had to accomplish i) to have for at least six months sustained LDA (defined in RA patients by the Disease Activity Score of 28 joints (DAS28)<3.2 and in axial SpA patients by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)<4 with one of these conditions: normal C-reactive protein (CRP) or delta-BASDAI>50%); ii) to be treated with the same TNF inhibitor throughout the entire study and iii) to have received treatment in both study periods.

Seventy seven patients (36 (46.7%) with RA and 41 (53.3%) with SpA) out of the total of 395 treated with Infliximab(Ifx), Adalimumab (Ada) or Etanercept (Etn) in the Rheumatology Department from La Paz University Hospital, met these inclusion criteria.

All RA patients fulfilled the 2010 or 1987 ACR (American Collegue of Rheumatology) revised criteria [19] and all SpA patients have axial involvement and fulfilled the New York revised criteria [24] or the ASAS (Ankylosing Spondylitis Assessment Study) group criteria [25]. Clinical activity was evaluated at baseline and every 6 months by the DAS28 in RA patients and by the BASDAI in SpA patients. BASDAI was used to evaluate SpA patients instead of ASDAS because no ASDAS values were available at the beginning of the study period. This was an observational study that did not require the approval of the Hospital Ethical Committee.

The tapering strategy consisted in a progressive interval prolongation (increasing the interval of Ifx and Ada administration by one week and of Etn administration by 3 days) and/or dose reduction (decreasing by 1 mg/kg until 3 mg/kg in SpA patients treated with Ifx) following the physician criteria based on clinical and serological markers CRP, erythrocyte sedimentation rate-ESR- and TNFi levels).

A flare was defined as an increase of the DAS28-ESR (a composite score measuring disease activity) greater than 3.2 plus a delta-DAS28 (related to pre-tapering DAS28) lower than -0.6 in RA patients and BASDAI ≥ 4 and delta-BASDAI ≤-2 (related to pre-tapering BASDAI) in SpA patients in at least one clinical visit during the study [20,26]. In the case of a flare, the concomitant therapy (disease-modifying antirheumatic drugs-DMARDS-, nonsteroidal anti-inflammatory drug -NSAIDS-, corticosteroids) could be intensified in both periods, but the TNFinhibitor therapy was only increased in the 2nd P.

Out of the 77 patients, 29 were treated with Ifx, 27 with Ada, and 21 with Etn. In the 1st P, Ifx was administered intravenously to RA patients at 3 mg/kg at 0, 2, 6 weeks and every 8 weeks thereafter; and to patients with SpA at 5 mg/kg at 0, 2, 6 weeks and every 8 weeks thereafter. Ada was administered at 40 mg/2 weeks and Etn at 50 mg/week following the drug labels. In the 2nd P, clinicians were allowed to increase the administration interval and/or reduce dose in case of Ifx if the patient was presenting LDA.

Blood samples were collected a maximum of 24h before the biologic drug administration for a subcutaneous TNF inhibitor or just before intravenous (i.v.) infusion for Ifx. Drug and ADA levels were measured at every visit for patients on Ifx treatment and every 6 months for patients on subcutaneous administration.

Measurement of drug and ADA concentration

Serum drug concentrations were determined by a capture enzyme-linked immunosorbent assay (ELISA), as described previously [27]. Cut-off values for positive drug levels were 10 ng/ml for Ifx, 5 ng/ml for Ada and 30 ng/ml for Etn. Serum ADA levels were assayed using a two-site (bridging) in-house ELISA [27,28] with a cut-off for positivity of 10 arbitrary units (AU)/ml for all anti-TNFinhibitor antibodies.

Evaluation of the dispensed amount of drug and cost analysis

The drug amount that each patient received was obtained from the database of the Pharmacology Department. We evaluated the mean dose delivered to the patient and the mean time elapsed between the dispensations in both periods of the study. With these data, the mean weekly dose was calculated. The cost of the treatment in each period was calculated taking the price of each medication at the end of 2012 into account.

Statistical analysis

Differences in baseline characteristics were assessed using Pearson's chi-square test and Fisher's exact test for ordinal variables. Continuous non-parametric data were compared between groups using the Mann-Whitney U test. Paired comparisons of parametric results were performed by Student's t-test and non-parametric results by the Wilcoxon matched-pairs test. p-values were considered significant when under 0.05. Statistics were performed using GraphPad Prism6 software (San Diego, CA, EEUU).

Results

Patient characteristics

A total of 77 patients, (36 RA and 41 SpA) were analyzed in this study. The baseline demographic and clinical characteristics are shown in Table 1. None of the patients discontinued the TNF inhibitor treatment during the study.

	Total	RA	SpA
Number of patients	77	36	41
Age, years *	56.31 (12.9)	60.4 (12.3)	53.4 (12.4)
Female, n (%)	40 (51.3)	27(73)	13 (31.7)
RF positive, n (%)	28 (37.1)	29 (78)	------
ACPA positive, n (%)	27 (35.0)	27 (75.0)	------
DAS28/BASDAI prior to TNFi**	------	4.6 (1.4)	5.1 (1.4)
Disease duration prior to TNFi*	10.00 (8.7)	10.5 (8.4)	9.5 (9.1)
Time on TNFi at inclusion*	2.44 (1.8)	2.92 (1.9)	1.99 (1.5)
DAS28 /BASDAI at inclusion**	------	2.32 (0.5)	1.88 (0.9)
Time on biologic treatment, 1st P*	2.09 (0.8)	2.36 (0.6)	1.78 (0.9)
Time on biologic treatment, 2nd P*	2.46 (0.3)	2.51 (0.3)	2.4 (0.2)
Co-therapy			
Only MTX use, n (%)	28 (36)	20 (54)	8 (19.5)
Only other DMARD use, n (%)	21 (27)	6 (16)	15 (36.6)
MTX and other DMARDS, n (%)	12 (15.5)	6 (16.6)	6 (14.6)
Monotherapy, n (%)	16 (20)	4 (11)	12 (29.3)
Concomitant Prednisone use, n (%)	28 (36.3)	16 (44.4)	12 (29)
(*): mean years (**): mean (sd) = mean (standard deviation)			

Table 1: Baseline characteristics of all patients in the follow-up cohort.

Comparison of disease activity before and during the tapering strategy

No differences were observed in the clinical activity of patients between the 1st P and the 2nd P, even when the TNF inhibitor subgroups were considered separately (Table 2). During the six years of the studied periods, 31 (39.7%) patients had one or more flares (18 RA and 13 SpA), and 4 of them (2 RA and 2 SpA) had a flare in both periods. No significant differences were found in the number of patients with flares, neither in the total number of flares between the 1st and the 2nd P.

	DAS28			BASDAI		
	1st P	2nd P	P	1st P	2nd P	p
Ifx, n=29	2.37(0.51)	2.31(0.76)	0.78	1.72(0.72)	1.75(0.88)	0.886
Ada, n=27	2.36(0.35)	2.35(0.33)	0.908	2.10(1.44)	2.00(1.13)	0.700
Etn, n=21	2.15(0.56)	2.38(0.55)	0.124	2.07(0.81)	2.19(0.79)	0.657
Total	2.28(0.47)	2.37(0.50)	0.200	1.88(0.95)	1.90(0.93)	0.910
*mean(sd)						

Table 2: Clinical activity of the RA patients, expressed using the DAS28 index, and the SpA patients, expressed using the BASDAI index. Baseline values are those at the beginning of the anti-TNF therapy.

Nevertheless, a higher tendency to have flares in RA patients treated with Etn was observed in 2nd P (Table A1). Among the three TNFi, the differences in the number of flares and percentage of patients who developed flares were also not significant (Table A1).

Drug administration

In the 2nd P, the interval of drug administration was higher for all TNFi (8.7 ± 1.4 weeks in the 1st P vs 9.85 ± 1.5 weeks in the 2nd P, p<0.001 for Ifx; 2.3 ± 0.63 weeks in the 1st P vs 3.1 ± 1.02 weeks in the 2nd P, p<0.0001 for Ada; 1.4 ± 0.56 weeks in the 1st P vs 2.16 ± 1.57 weeks in the 2nd P, p<0.05 for Etn) (Figure 1A).

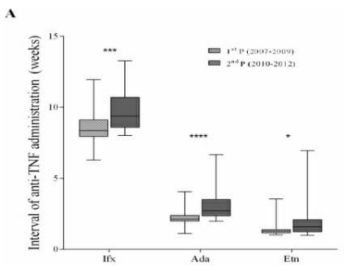

Figure 1A: Drug administration intervals in the 1st P (2007-2009) vs the 2nd P (2010-2012) for all three TNF inhibitors. Drug administration intervals are expressed in weeks. *p<0.05, ***p<0.001, ****p<0.0001"

A standard and stable dose was administered to every patient in both periods except for SpA patients treated with Ifx wherein the administered dose per patient was lower in the 2nd P (4.5 ± 0.75 mg/kg in the 1st P vs 4.1 ± 0.82 mg/kg in the 2nd P, p<0.05). Due to the longer interval of the administration in the 2nd P, the weekly mean amount of drug received per patient in this period was significantly lower (37.72 ± 12.58 mg/week during the 1st P vs 30.94 ± 10.76 mg/week during the

2nd P, p<0.001 for Ifx; 18.31 ± 4.84 mg/week during the 1st P vs 14.19 ± 3.68 mg/week during the 2nd P, p<0.0001 for Ada; and 37.78 ± 8.28 mg/week during the 1st P vs 30.68 ± 12.41 mg/week during the 2nd P, p<0.05 for Etn) (Figure 1B).

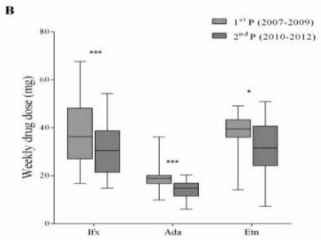

Figure 1B: weekly drug dose in the 1st P (2007-2009) vs the 2nd P (2010-2012) for all three TNF inhibitors. The weekly drug dose is expressed in mg. Box plots show the 25th and 75th percentiles, and the horizontal solid lines within the boxes indicate the means. *p<0.05, ***p<0.001, ****p<0.0001.

Hence, the overall yearly amount of drug received per patient (mg/year/patient) was lower in the 2nd P for all TNFi. The given amount of Ifx (taking each patients' body weight into account) was 1,967 ± 656 mg/year in the 1st P vs 1,613 ± 561 mg/year in the 2nd, p<0.001, whereas the given amount of Ada and Etn (calculated as the number of administered syringes x mg/syringe) was: 955 ± 253 mg/year in the 1st P vs 740 ± 192 mg/year in the 2nd P, p<0.0001 for Ada; and 1,970 ± 432 mg/year in the 1st P vs 1,600 ± 647 mg/year in the 2nd P, p<0.05 for Etn) (Figure 2A).

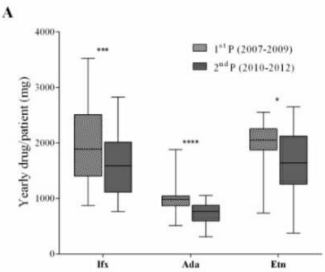

Figure 2A: Annual amount of drug consumed per patient for each TNFi in the 1st P (2007-2009) vs the 2nd P (2010-2012), expressed in mg/year/patient. *p<0.05, ***p<0.001, ****p<0.0001.

The amount of drug consumed decreased by 18% for Ifx, 23% for Ada and 19% for Etn in the 2nd P, resulting in an average saving of 20% in the amount of drug per year. Based on the prices of these drugs in our hospital at the end of 2012, the cost/year for each patient was significantly reduced in the 2nd P for all three TNFi (Figure 2B); the estimated total cost saving was approximately €153,798/year, with a mean saving/patient/year of €1,715 for Ifx, €2,580 for Ada, and €1,638 for Etn.

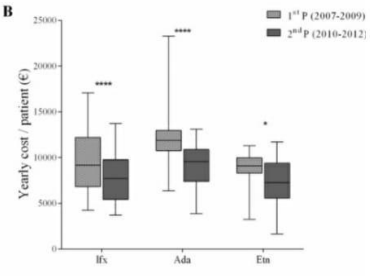

Figure 2B: Annual cost per patient for each TNFi in the 1st P (2007-2009) vs the 2nd P (2010-2012), expressed in €. Box plots show the 25th and 75th percentiles, and the horizontal solid lines within the boxes indicate the means. * p<0.05, *** p<0.001, ****p<0.0001.

Circulating drug levels and immunogenicity

During the 2nd P, serum trough drug levels were significantly lower. Mean+SD serum drug levels in the 1st P versus serum drug levels in the 2nd P were 3.2 ± 2.5 µg/ml vs 1.8 ± 1.5 µg/ml (p<0.0001) for Ifx; 5.5 ± 2.8 µg/ml vs 3.1 ± 2.1 µg/ml (p<0.0001) for Ada; and 1.8 ± 1.1 µg/ml vs 1.3 ± 0.8 µg/ml (p<0.05) for Etn (Figure 3).

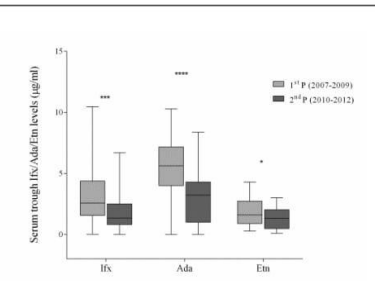

Figure 3: Serum trough drug levels in the 1st P (2007-2009) and in the 2nd P (2010-2012) for the three TNF inhibitors. Box plots show the 25th and 75th percentiles, and the horizontal solid lines within the boxes indicate the means. *p<0.05, ***p<0.001, ****p<0.0001

The appearance of immunogenicity was not higher in the 2nd P (3 patients in the 1st P vs 7 patients in the 2nd P) and mean antibody levels in the 10 patients (17 samples) were very low (anti-Ifx antibodies: 18 ± 13 AU/ml; anti-Ada antibodies: 12 ± 8.7 AU/ml).

Discussion

In the present study we analyzed routine clinical practice over six years in a cohort of 77 patients and we compared the clinical course of the same patients before and after a tapering strategy. The results of this study showed that a tapering strategy of TNFi may be performed in patients with a sustained LDA without relevant changes in the clinical outcome, resulting in remarkable cost savings.

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ARTICLE 13

TITLE: "*Anti-TNF dose and anti-drug antibody levels in rheumatic and psoriasis patients: economic repercussion*"

JOURNAL: Eu J Clin Pharm 2015; 17(2): 95-104(146)

AUTHORS: González Fernández Maria Ángeles, Moreno Ramos Francisco, Varela Hugo Adolfo, **Plasencia Chamaida**, Pascual Salcedo Dora, Herranz Pinto Pedro, Herrero Ambrosio Alicia.

PATIENTS AND METHODS:

This is an ambispective observational study (2009-2012: without optimization strategy and from 2013: with optimization strategy) including patients with rheumatic diseases or psoriasis under biological therapy and in whom an optimization strategy was started. All optimized patients sustained a good clinical response.

Pharmacist reviews the prescriptions of all the patients treated with biological therapies when they are dispensed in the Pharmacy Department. Dosage regimens for all patients were checked.

A number, corresponding to the number of months that the amount of medication given to the patient would cover, was given to every dispensing process. This transformation is necessary in order to obtain the values of certain, parameters such as dispensed-patient-month, average-dispensed-patients and annual cost per average-patient established by the Healthcare System of the Community of Madrid.

Calculation of the dispensed-patient month, average-dispensed-patient and annual cost per average-patient

Annual cost per average-patient= Aggregate cost of biological drug/Mean dispensed-patient.

Aggregate cost was defined as the aggregate expenditure by average book price.

Annual average-dispensed patient was defined as the average of the dispensed-patient-months that have received treatment during the year.

Calculation of the annual costs per average-patient under biological treatment and per drug.

The aggregate-cost per average patient per year was calculated for each of the pathologies included in this study.

The statistical analysis consisted in calculating the statistical significance with Student´s test of the difference of proportions.

Finally, annual results obtained per patient in 2013 were compared to those calculated in previous years, and to the cost-per-drug for pathology in 2013. Thus, we were able to analyze the economic impact of the optimization of biological treatments.

ORIGINAL

ANTI-TNF DOSE AND ANTI-DRUG ANTIBODY LEVELS IN RHEUMATIC AND PSORIASIS PATIENTS: ECONOMIC REPERCUSSION

González Fernández María Ángeles, Moreno Ramos Francisco, Varela Hugo Adolfo, Plasencia Chamaida, Pascual Salcedo Dora, Herranz Pinto Pedro, Herrero Ambrosio Alicia

ABSTRACT

Introduction: In our hospital, the detection of anti-TNF and anti-drug antibody levels are used in clinical practice and can aim the clinicians to provide a safe and an efficient therapy.

Objective: To analyse the economic impact of the biological therapy optimization in patients with rheumatic and psoriasis by monitoring the drug and anti-drug antibody serum levels.

Method: Ambispective observational study. The retrospective study period extended from 2009 to 2012 and includes the results without optimization. The prospective study period started in 2013 and includes financial results with doses and administration intervals optimized. Drugs most frequently implicated were infliximab, etanercept and adalimumab.

Results: We checked doses and administration intervals of 449 adults with rheumatic diseases (41.8% under optimized regimen) and 167 patients with psoriasis (38.9% under optimized regimen) in 2013. The annual cost per average patient decreased by €1,345 in arthritic diseases and by €1,417 in psoriasis compared to the previous year.

Conclusion: Optimized treatments are more efficient, leading to a reduction in the annual costs per average patient. The access to the clinical information of patients and the integration of pharmacists into multidisciplinary teams alongside provide a better high-quality pharmaceutical care, and evaluation of economic and clinical results.

Anti-TNF – Antibodies – Monitoring – Optimization – Costs – Rheumatic – Psoriasis

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RESUMEN

Introducción: La detección de niveles en plasma de anti-TNF y anticuerpos anti-TNF es una práctica habitual en nuestro hospital, que orienta al clínico en la toma de decisiones, proporcionando una terapia segura y eficiente.

Objetivo: Analizar el impacto económico de la optimización de las terapias biológicas tras la monitorización de niveles plasmáticos de fármacos biológicos y anticuerpos anti-TNF en pacientes con enfermedades reumáticas y psoriasis.

Método: Estudio observacional ambispectivo. El estudio retrospectivo se extiende desde 2009 a 2012 e incluye los resultados económicos de los tratamientos con pautas no optimizadas. El estudio prospectivo comenzó en 2013 y refleja los resultados económicos incluyendo las dosis e intervalos de administración optimizados. Los medicamentos implicados en su mayoría fueron infliximab, etanercept y adalimumab.

Resultados: Se revisaron las dosis e intervalos de administración de 449 pacientes adultos con artropatías (41,8% optimizados) y 167 pacientes con psoriasis

(38,9% optimizados) tratados en 2013. El coste anual del paciente medio en 2013 descendió respecto al año anterior en 1.345 € en pacientes con artropatías, y en 1.417 € en pacientes con psoriasis.

Conclusión: Los tratamientos optimizados son más eficientes, produciéndose un descenso en el coste anual del paciente medio. El acceso a la información clínica del paciente y la integración del farmacéutico a los equipos multidisciplinares facilitan una mejor atención farmacéutica al paciente, su seguimiento fármaco-terapéutico y la evaluación económica de los resultados.

Anti-TNF – Anticuerpos – Monitorización – Optimización – Coste – Reuma – Psoriasis

INTRODUCTION

The introduction of biological agents, such as antibodies that antagonize the tumour necrosis factor (TNF) have improved the clinical outcome in refractory patients to conventional therapy, inducing long-term remission in immune-inflammatory diseases: rheumatic diseases as rheumatoid arthritis (RA) and spondyloarthritis (SpA), inflammatory bowel diseases and psoriasis (SD), specifically psoriasis.[1-3]

Clinicians regularly decrease the drug dosages once a therapeutic goal has been reached and consolidated to avoid the potentially serious adverse effects reported with these drugs. The principal reason for optimization is the need to improve the risk/benefit ratio.

However, there are patients that do not respond at all (primary response failure) or show clinical relapse after initial improvement (secondary response failure).[4, 5] At least in part treatment failure has been attributed by the development of anti-drug antibodies (ADA).[6, 7]

Infliximab (Ifx) and adalimumab (Ada) can have a faster clearance due to the formation of anti-drug neutralizing antibodies (ADA)[7, 8] resulting in loss of efficacy and appearance of side effects such as infusion-related reactions.[9] Serum drug levels are inversely correlated with the presence of ADA and loss of clinical response.[10] At present, in the case of etanercept (Etn), ADA have not been detected by commonly used ELISA assays.[11]

However, treatment monitoring can not be restricted to the antibody determinations, indeed, most authors agree in the need of monitoring both drug levels and ADA formation as the best rationale for treatment optimization. In this setting, it has been argued that immunogenicity monitoring can be useful to differentiate patients who will benefit from a change in anti-TNF therapy from those who show no primary response. Therefore, sequential determination of drug and ADA levels can be used to customize treatment, helping to avoid unnecessary therapies and to provide a safe and an efficient therapy.[12]

Determination of serum drug levels (Ifx, Ada and Etn) and ADA (anti-Ifx y anti-Ada) is a usual practice in the Immunology Unit in our hospital.[13, 14] The Departments of Rheumatology and Dermatology started the monitoring Ifx levels and antibodies to Ifx appearance from 2011 and 2012 respectively. The remaining anti-TNF drug and ADA began in 2013 in both departments. These determinations have been used in the clinical monitoring of patients, helping clinicians make therapeutical decisions to optimize the biological therapy, by extending the dosing interval and/or by reducing the dose of Ifx administered intravenously.

In this study, we present data of doses and administration intervals optimized on patients treated with anti-TNF as well as the financial consequences of the optimization strategy, based on the combination of clinical parameters with drug and ADA levels monitoring, classified by patient and by drug, in rheumatic diseases (RA and SpA) and SD.

METHOD

This was an ambispective observational study. The retrospective study period extended from 2009 to 2012, including financial results with doses and administration intervals without optimization. The prospective study period extended from 2013 on and includes financial results with the optimization strategy (progressive dose decrease and/or interval elongation). All optimized patients maintained a good clinical response, as shown in clinical parameters, as a requisite for dose reduction or administration intervals increase.

Pharmacist reviews the prescriptions of all the patients treated with biological therapies when they are dispensed in the Hospital Pharmacy Department. The three next most frequently implicated drugs were infliximab, etanercept and adalimumab. Dosage regimens for all patients diagnosed with RA, SpA and SD were reviewed. Paediatric patients under 18 years of age and diagnosed with SpA were also included in our study. These data are recorded in our software for drug prescribing and dispensing to outpatients (FarmaTools 2.5 Dominion).

A number, corresponding to the number of months that the amount of medication given to the patient would cover, was given to every dispensing process. This transformation is necessary in order to obtain the values of certain parameters such as dispensed-patient-month, average-dispensed-patient and annual cost per average-patient established by the Healthcare System of the Community of Madrid.

Calculation of the dispensed-patient-month, average-dispensed-patient and annual cost per average-patient.

Annual cost per average-patient = [Aggregate cost of biological drugs (1) / Mean-dispensed-patient (2)]

TABLE 1. Demographic characteristics of patients.

Parameters	*2013 No. of patients (%)*	*2012 No. of patients (%)*	*p*
Reumathic patients			
— IV Therapy	314 (100)	299 (100)	0.096
• Women	177 (56.4)	184 (61.5)	
• Men	137 (43.6)	115 (38.5)	
— SC Therapy	135 (100)	139 (100)	0.488
• Women	95 (70.3)	97 (69.7)	
• Men	40 (29.7)	42 (30.7)	
Total adults	449	438	
— Paediatric	79 (100)	78 (100)	0.481
• Women	59 (74.6)	58 (74.3)	
• Men	20 (25.4)	20 (25.7)	
Psoriasis patients			
— All patients	167 (100)	173 (100)	0.387
• Women	50 (30.1)	71 (41.3)	
• Men	117 (69.9)	102 (58.7)	

Statistical Significance p <0,05

(1) Aggregate cost: aggregate expenditure by average book price.

(2) Annual average-dispensed-patient: it is defined as the average of the dispensed-patient-months* that have received treatment during the year.

* Patient-dispensed-month (number of patients that have received medication multiplied by the number of months dispensed to each patient over the period analysed). It refers to the medication dispensed to the patients expressed in months, namely the sum of the number of months that each patient has been exposed to the medication dispensed during one month.

An Excel template is available in order to help introduce the necessary data to calculate this indicator.

Calculation of the annual cost per average-patient under biological treatment and per drug. In the same manner as described above, aggregate-cost per average patient per year was calculated for each of the pathologies analysed. In the case of patients with SpA, costs are calculated differentiating between adult and paediatric patients.

The statistical analysis consisted in calculating the statistical significance with Student's test of de difference of proportions (statistically significant values $p < 0.05$).

Lastly, annual results obtained per patient in 2013 were compared to those calculated in previous years, and to the cost-per-drug for pathology in 2013. Thus, we were able to analyse the economic impact of the optimization of biological treatments.

RESULTS

In 2013, there were 449 adult patients with rheumatic diseases and 167 with psoriasis. In 2012 there were 438 adult patients with rheumatic diseases and 173 with psoriasis. Demographic characteristics of the patients are shown in Table 1. There were no observed statistically significant differences between groups in 2012 vs. 2013.

Rheumatic diseases. Doses and administration intervals corresponding to 449 adult patients diagnosed with rheumatic diseases were revised in 2013, 188 of whom (41.8%) were following an optimized dosage regimen. The distribution of patients was as follows:

— 209 adult patients with RA:
  • 145 patients treated with a subcutaneous biological agent: 67 under optimization strategy (46.2%).
  • 64 patients with intravenous biological agent: 26 patients treated with Ifx (23 patients with administration intervals optimized), 36% of patients under optimization strategy.

— 240 adult patients SpA:
  • 176 patients treated with a subcutaneous biological agent: 68 following optimization strategy (38.6%).
  • 64 patients treated with Ifx: 15 patients under a 3 mg/Kg dosage regimen, and 15 patients under a 4 mg/Kg dosage regimen. A total of 46.8% of patients under optimization strategy.

As for paediatric SpA patients, the dosage regimens corresponding to 79 children were revised. Those regimens were prescribe depending on body weight and followed recommendations specified on the data sheet of each drug, except for nine cases in which Ada was prescribed as a weekly dose.

Psoriasis. Treatments for 167 patients with SD were revised in 2013, 65 of whom (47.9%) were under optimization strategy.

TABLE 2. Doses and administration intervals with the optimization strategy.

Reumathois Arthritis (RA) *Doses and administration interval*	*No. (%)*	*Spondyloarthritis (SPA)* *Doses and administration interval*	*No. (%)*	*Psoriasis* *Doses and administration interval*	*No. (%)*
Etanercept 50 mg/10 days	15 (16.67)	Etanercept 50 mg/15 days	14 (14.29)	Etanercept 25 mg/wk	6 (9.23)
Etanercept 50 mg/15 days	9 (10)	Adalimumab 40 mg/3 wk	13 (13.27)	Etanercept 25 mg/10 days	3 (4.62)
Adalimumab 40 mg/4 wk	7 (7.78)	Etanercept 50 mg/10 days	7 (7.14)	Etanercept 50 mg/15 days	5 (7.69)
Adalimumab 40 mg/3 wk	7 (7.78)	Etanercept 25 mg/10 days	7 (7.14)	Etanercept 50 mg/10 days	10 (15.38)
Etanercept 50 mg/3 wk	5 (5.56)	Etanercept 25 mg/wk	7 (7.14)	Adalimumab 40 mg/4 wk	2 (3.08)
Certolizumab 200 mg/3 wk	4 (4.44)	Adalimumab 40 mg/4 wk	5 (5.1)	Adalimumab 40 mg/3 wk	10 (15.38)
Adalimumab 40 mg/5 wk.	4 (4.44)	Adalimumab 40 mg/5 wk	3 (3.06)	Adalimumab 40 mg/30 days	1 (1.54)
Etanercept 25 mg/10 days	2 (2.22)	Etanercept 50 mg/18 days	2 (2.04)	Ustekinumab 45 mg/4 mo	12 (18.46)
Etanercept 25 mg/wk	2 (2.22)	Etanercept 25 mg/2 wk	2 (2.04)	Ustekinumab 45 mg/5 mo	3 (4.62)
Certolizumab 200 mg/4 wk	2 (2.22)	Etanercept 50 mg/3 wk	1 (1.02)	Ustekinumab 45mg/6 mo	1 (1.54)
Adalimumab 40 mg/30 days	2 (2.22)	Etanercept 50 mg/30 days	1 (1.02)	Infliximab 3 mg/kg/8 wk	8 (12.31)
Adalimumab 40 mg/6 wk	2 (2.22)	Etanercept 25 mg/18 days	1 (1.02)	Infliximab 4 mg/kg/8 wk	4 (6.15)
Etanercept 50 mg/18 days	1 (1.11)	Etanercept 25 mg/3 wk	1 (1.02)		
Etanercept 25 mg/3 wk	1 (1.11)	Adalimumab 40 mg/18 days	1 (1.02)		
Adalimumab 40 mg/7 wk	1 (1.11)	Adalimumab 40 mg/26 days	1 (1.02)		
Adalimumab 40 mg/18 days	1 (1.11)	Adalimumab 40 mg/24 days	1 (1.02)		
Adalimumab 40 mg/8 wk	1 (1.11)	Adalimumab 40 mg/30 days	1 (1.02)		
Adalimumab 40 mg/7 wk	1 (1.11)	Infliximab 3 mg/kg/8 wk	15 (15.31)		
Infliximab 3 mg/kg/8 wk	23 (25.56)	Infliximab 4 mg/kg/8 wk	15 (15.31)		
Total	90 (100)	Total	98 (100)	Total	65 (100)

No.: Number of patients; wk: weeks; mo: months.

— 140 patients treated with a subcutaneous biological agent, 53 of whom were optimized treatment regimens (37.8%).
— 27 patients under treatment with Ifx 12 patients were following an optimized treatment (44.4%), eight of whom treated with doses of 3 mg/Kg.

The different optimized dosage regimens and their proportion at the end of 2013, classified by pathology, are included in Table 2. In this table, dosage regimens corresponding to paediatric patients do not appear because the prescribed doses are adjusted by age and body weight, except in the case of Ada, which some patients received as a fixated 20-30 mg weekly dose. Appear optimized dosage regimens corresponding to certolizumab and ustekinumab patients too, all based on clinical outcomes, not based on antibody or drug levels determinations.

Regarding the overall cost (in Euros) of the treatment with biological agents, Fig. 1a and 1b show the evolution of expenditure in these treatments in the Departments of Rheumatology and Dermatology from 2009 to 2013.

Fig. 1a and 1b show the cost/patient/year, including the percentage reduction in cost per patient compared to previous years, in the Departments of Rheumatology and Dermatology respectively.

The evolution of the results per average-dispensed-patient for both departments, from 2009 to 2013, is shown in Fig. 2a and 2b.

Annual costs per average patient and per drug in 2013 are shown in Table 3, distinguishing between patients with RA, patients with SpA (adults and children) and patients with SD.

Table 4 reflects the trend in percentage change of pharmaceutical expenditure in biological drugs for both groups of diseases over time.

Table 5 shows the percentage distribution of patients under an optimized therapy for pathology.

DISCUSSION

Prior to determination of biological drugs and ADA serum levels, clinicians are left with only a few choices when drug failure, all based on clinical outcome. When a failure is detected in these patients, clinicians usually make empirical decisions, intensifying the current therapy regimen, switching to another anti-TNF or switching to a different class of drug. The disadvantage of choosing new biological therapies based only in clinical parameters, in patients with a previous failure, can lead to erroneous therapeutic decisions with the consequent clinical worsening of patients and the increase of financial costs. In the department of Rheumatology and Dermatology of our hospital, the optimization protocols for biological therapy for the decision makers are based on clinical parameters (DAS 28, EULAR, BASDAI, ASDAS, PASI), analytical parameters (VSG, PCR), and determination of drug levels and ADA. The use of all of them allows the clinicians to individualize therapy.[13]

In the present study, a relevant decrease in costs has been detected after the optimization of biological therapies by using the drug and ADA levels, monitored together with the clinical parameters.

The serum drug level determinations offers the possibility of tailoring therapy according to individual needs and it aims at reducing delays in effective treatment[15] or

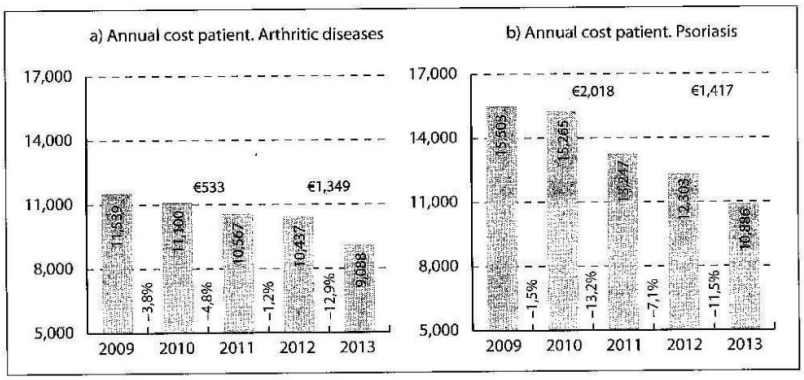

FIG. 1. Annual cost per average-patient evolution.

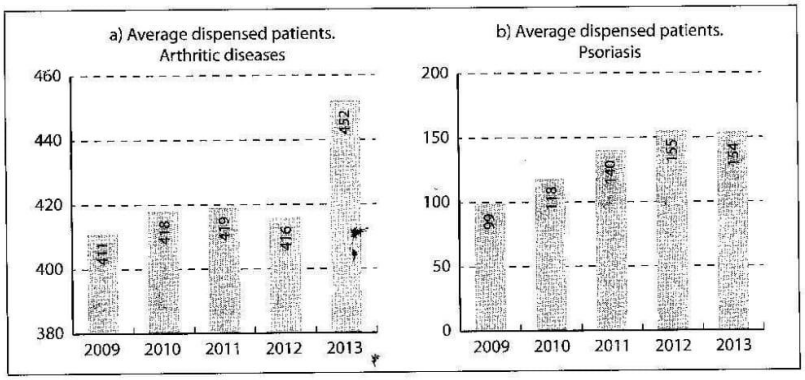

FIG. 2. Average dispensed patient evolution.

modifying treatment regimens. It is of note that all optimized patients maintained a good clinical response, as shown in clinical parameters, as a requisite for dose reduction.

Serum drug levels are inversely correlated with the presence ADA and with the clinical response.[6, 10, 16] Testing for anti-drugs antibodies may also help to clinicians to be caution about the risk of potentially dangerous infusion-related reactions,[9, 17, 18] which have been related to the development of anti-Ifx antibodies. The development of antibodies to the specific monoclonal IgGs may also lead to an effector mechanism involving complement activation and production of anaphilotoxins, which may lead to severe side effects.[18] Therefore, determination of drug levels and ADA has the potential to improve the cost-effectiveness of these expensive therapies.

The results of our study show a decrease in the costs of these therapies after optimizing treatments by monitoring anti-TNF drug and ADA serum levels.

Data regarding costs per annual average-patient diagnosed with a rheumatic disease show a pronounced decrease in two different periods: 2010-11 (–4.8%), and 2012-13 (–12.9%). Those percentage decreases translate into –€533 and –€1,349 per average-patient and year respectively (Fig. 1a). That reduction in costs occurs despite the fact that data regarding average-dispensed-patient for the period 2010-11 is similar, and that for the period 2012-13 increases from 416 to 452 months (Fig. 2a). The implementation of the Royal Decree-Law 4/2010, enforced in June 2010, resulted in a decrease of PTR of 7.5% for this type of medication.[19] This Royal Decree-Law is the main cause for the decline in costs for that period from 2010 to 2011, while the decline in costs for the period 2012-2013 can be mainly explained by the optimization of treatments with dose medications by adjusting dosage and administration intervals.

We consider the variable «annual cost per average-patient» valid for this type of calculations, since it is independent of several other factors such as: 1) the number of patients; 2) time periods in which patients discontinue therapy due to lack of treatment compliance, relapses or surgical operations; 3) the time in the year in which patients start treatment; 4) the number of months of treatment that will be covered by the amount of medication dispensed. Moreover, this dynamic variable includes changes in dosage and treatment regimens for a single patient occurred throughout the year.

TABLE 3. Annual cost per average patient and per drug in 2013.

Drug	*RA*	*SPA Adults*	*SPA Paediatrics*	*Psoriasis*
Adalimumab	9,017	9,864	14,806	10,631
Etanercept	9,109	8,490	6,732	10,292
Certolizumab	8,408	–	–	–
Golimumab	10,287	10,973	–	–
Ustekinumab	–	–	–	10,793
Infliximab	5,812	8,897	–	10,090
Tocilizumab	9,442	–	–	–
Abatacept	10,007	–	–	–
Rituximab	5,002	–	–	–

RA: Reumathois Arthritis; SPA: Spondyloarthritis.

TABLE 4. Percentaje chage of pharmaceutical expenditure.

	2010-11	*2011-12*	*2012-13*
Arthritic Diseases			
— Adalimumab	–5.5	–5.5	–16.4
— Etanercept	–4.8	–5.1	–9
— Infliximab	–19.5	–14.5	–15.4
— Tocilizumab	142	51.2	–2.3
— Rituximab	–18.3	–14.7	–8.6
— Abatacept	53.1	21.7	–26.1
— Certolizumab	1,673	96.8	4
— Golimumab	0	45	152.3
Skin Diseases			
— Adalimumab	7.6	18.7	–6.6
— Etanercept	–12.4	–4.9	–13.7
— Infliximab	6.7	–5.2	–3.4
— Golimumab	20.3	5.2	–10.5

Units: Percentage increase (%).

Regarding patients with psoriasis, when we analyse the trend in the annual cost per average patient, there also appear two periods of pronounced decline in costs. The first period of decline corresponds to 2010-11 (–13.2%, –€2,018) and can be explained by the afore-mentioned official discount of 7.5% and other discounts in price that were implemented in 2011. The second period of decline corresponds to 2012-13 (–11.5%; €–1,417) and, again, can be attributed to the optimization of therapies (Fig. 1b). From 2009 to 2012, an upward trend has been observed regarding the values of average-dispensed-patient, trend that persists in 2013.

We believe the fact that the decrease of the annual cost per patient between 2010 and 2012 has been more substantial for patients diagnosed with psoriasis than for those suffering from rheumatic diseases is because the discounts, in the case of patients suffering from rheumatic diseases, are watered down because the number of drugs available is greater than for the treatment of psoriasis. The fact that the decrease in the annual cost per patient in the period 2010-2012 has been more substantial for patients diagnosed with psoriasis that for those with rheumatic diseases is because there are more drugs available for the treatment of rheumatic diseases, so the afore-mentioned discounts applied to certain drugs become diluted.

If the percentage increases of expenditure in biological medications are analysed, it is evident that there existed a decrease in the amount of Ifx dispensed to patients with rheumatic diseases from 2011 on, and to patients diagnosed with psoriasis from 2012 on, coinciding with the start of the monitoring of this drug in both groups of patients (Table 4). Nevertheless, that decrease is less pronounced for the group of psoriasis, since the number of patients undergoing treatment with Ifx is lower that for the group with rheumatic diseases. With regard to the decrease in expenditure related to Etn and Ada for both groups of pathologies, this decline is more pronounced in the period 2012-2013, coinciding with the beginning of the optimization of treatments with both medications in 2013.

Existing publications of works in our environment [20-22] that analyse the economic impact of biological treatments in RA. The first study concludes that the drug with a lower

TABLE 5. Percentage distribution of patients under optimization strategy.

	RA *No. of patients (%)*	*SPA* *No. of patients (%)*	*Psoriasis* *No. of patients (%)*
Etanercept	35 (38.9)	43 (43.9)	24 (36.9)
Adalimumab	26 (28.9)	25 (25.5)	13 (20)
Inflixmab	23 (25.6)	30 (30.6)	12 (18.5)
Cerlolizumab	6 (6.7)		
Ustekimumab			16 (24.6)
TOTAL	90 (100)	98 (100)	65 (100)

RA: Reumathois Arthritis; SPA: Spondyloarthritis.

cost/patient/year, under established practice, is Etn, followed by Ifx and, lastly, Ada. Our study differs in that the less expensive drug is Ifx (–42.4% as compared to the afore-mentioned study). Etn and Ada have a similar annual cost per patient in our study, but are a 5.05% and a 23.8% less expensive respectively when compared to the previous study. Probably, the results of the first study are not truly comparable to those of our study, as the afore-mentioned work was conducted from 2006 to 2010 and it is very likely that treatments were not optimized, not even empirically.

Another study[21] shows results of annual cost per patient that are higher than ours by 54.7% in the case of Ifx, 18.9% for Etn, and 23.5% regarding Ada. A third study[22] also provides costs related to those treatments that are much higher that the costs per patient in our study.

The annual cost per patient of treatment with Etn or Ada in our study is higher for patients with psoriasis because there are more patients under an intensified regimen and that follow that therapy for a longer period of time than in the case of patients with rheumatic diseases (Table 3). Moreover, there is an induction dose of Ada in the case of psoriasis that there does not exist for the treatment of rheumatic diseases.

If costs of treating paediatric patients are compared to those of adult patients with SpA (Table 3), the cost of a treatment with Etn is lower for paediatric patients since the doses are adjusted by body weight. Nevertheless, the annual cost per paediatric patient of a treatment with Ada is substantially higher than the annual cost per adult patient. This is explained by the fact that there are cases in which Ada is prescribed to paediatric patients at a weekly dose of 20-30 mg, after monitoring serum drug levels. Manufacturing companies have only marketed the 40 mg syringe, so paediatric patients undergoing this kind of weekly dosage regimen need twice as much syringes to cover treatment for a month compared to the biweekly regimen approved in the data sheet of Ada.

In our cohort of patients, it was observed that most patients under optimization strategy were treated with Etn (Table 5).

We believe that our study has some limitations. One of those is that financial results do not correlate with clinical results such as PASI 75-90 or DAS28 or BASDAI, something that would have strengthen our results. Nevertheless all the patients optimized reached a stable therapeutic goal with the dose that would provide adequate disease control once inflammatory activity has been resolved. Another limitation is the absence of an analysis of avoided costs, since we do not have data regarding rheumatology patients in remission, dermatology patients cured, or permanent discontinuation of treatments due to ineffectiveness.

It is important to mention that all data on the costs per patient obtained after monitoring and treatment optimization are dynamic data, that is, they change over time depending on clinical practice and the characteristics of each patient and on the price of the drug. Thus, the pharmacist's role in the calculation of such costs is essential to establish the most efficient clinical practice in each situation.

Finally, we may conclude that monitoring biological drug serum levels and ADA in immune-inflammatory diseases allows adjustment of the dosage regimens of such drugs according to patient's needs. As our study has proved, resulting treatments after optimization are more efficient, leading to a significant decrease in the annual cost per average-patient for the diseases considered, and also a global reduction in the expenditure on such medications.

Pharmacists can take a more efficient role in the pharmacotherapeutic process in patients treated with biological drugs in inflammatory diseases when they review prescriptions with relevant patient medical information such as optimized biological drugs and are integrated in the clinical team.

This calculation is a dynamic parameter that should be measured periodically, since it may vary depending on clinical practices and the degree to which treatments are optimized. CP

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DISCUSSION

In the present work, we have shown the results from a deep research about a very important topic: the immunogenicity of biologics and its clinical relevance in rheumatic patients. From the beginning of the biological treatment, it was seen that a group of patients had primary or secondary inefficacy. As a result of the monitoring of drug and ADA levels, we have been able to see a huge variability of drug concentrations in patients with the same pathology under the same biologic regimen.

Our initial studies demonstrated the correlation between serum trough drug and ADA levels with clinical outcomes in RA and SpA patients from the biologic cohort from the La Paz University Hospital(73,96,103). Later, we investigated if serum trough drug and ADA monitoring could be useful to make therapeutic decisions in patients under TNFi treatment who develop a drug failure(118,141).

Currently, it is more common to have patients with sustained low disease activity under long-standing biologic therapy. The tapering strategy of biologics (decreasing dose or increasing the interval of administration) seems feasible and has been more frequently used in the last years(142). One of the main concerns for clinicians is the appearance and management of flares in patients under a tapering strategy. We presented two observational studies comparing the tapering strategy to the standard therapy regimen in RA and SpA patients in clinical practice(143,144). These works are relevant because they show that a progressive tapering strategy is feasible in clinical practice because it entails similar clinical outcome as patients under a standard therapy regimen(143,144).

Finally, another concern for our health system is the cost of biopharmaceuticals in patients with rheumatologic diseases for two obvious reasons: its high cost and because its use is increasing due to the prevalence of these diseases. Referring to this topic, we carried out two studies demonstrating that adequate clinical monitoring together with tools such as drug monitoring can help optimization strategies to be carried out more safely with a significant reduction of costs(145,146).

Association between serum drug and ADA levels with clinical outcomes

As previously reported, both in RA and SpA patients treated with Ifx, we have found that serum Ifx trough levels are inversely correlated with clinical response. In addition, undetectable serum Ifx levels were associated with ATI appearance(25,48,104,147). Other authors have also shown that ATI+ patients were more often classified as non-responders than ATI-(68,71,93,94). These studies have shown this relationship in shorter follow-up periods compared to ours, which included follow-up of up to 4 years. A study with a comparable duration time is the one performed in a Dutch RA cohort of 272 patients treated with Ada(47). In this population, they observed similar correlation between ADA statuses and the clinical response(47). Furthermore, another work in SpA patients treated with Ada observed a significant inverse correlation between Ada levels and clinical activity measured by ASDAS and with ADA titres(76).

Drug survival is another indirect parameter related to clinical efficacy that has been evaluated in several studies. Most publications observed that ADA+ patients have more frequent discontinuations and a less biological drug survival(47,71). In our cohort under Ifx therapy, the proportion of ATI+ patients who discontinued the drug was high, being 82% in RA and 73% in SpA patients. Moreover, the Ifx survival was significantly lower in patients with detectable ATI in comparison with patients without ATI(73,96).

As has been explained in the introduction, not all biological drugs have the same immunogenic ability and structural differences can influence both the production of antibodies and their ability to be detected with currently available assays(54,56,61,63). In patients treated with Etn, Goli or Tcz, the frequency of ADA is low or practically nonexistent(54,85,102,148,82,149,81,84,89). However, a clear correlation has been seen between serum trough drug levels and clinical outcomes. In a cohort of 292 RA patients treated with Etn, serum Etn levels were significantly higher in good responders as compared to both moderate and EULAR non-responders after 6 months of therapy(95). Kneepkens et al. observed in an AS cohort of 162 patients that Etn levels were significantly higher in patients with ASDAS<2.1compared to ASDAS≥2.1

patients(148). When patients were categorized into quartiles according to Etn levels, the lowest quartile (Etn<1.80 mg/L) comprised 35% of all patients with ASDAS≥2.1 while the highest quartile comprised only 14%. No antibodies against Etn were detectable in this study(148). Our group also carried out studies with less immunogenic TNFi such as Goli in RA patients(102). With Goli, an inverse correlation between clinical activity and serum drug levels was noted(102). After stratifying patients into groups according to drug levels, inactive patients appeared in the highest quartiles(102). We only found that 3 out of 37 patients were ADA+ and all of them discontinued therapy due to inefficacy(102). The proportion of patients who developed ADA during Goli treatments varied between 2.1%-15.2% (58,149,89). Chen et al. found the highest proportion of ADA+ RA patients under Goli treatment (15.2%) possibly due to the lower number of patients taking concomitant immunosuppressive drugs like MTX, the lower dose of MTX, differences on the assays and the small sample size(89).

Tcz is a humanized antibody that competitively inhibits soluble and membrane-bound IL6R(150). For the TNFi therapy, it has been shown that immunogenicity can have a profound effect on the PK(21,22,24,25,27,49,54,57,151). Nevertheless, the PK of Tcz appears to be different. In our study, only 1 patient had detectable antibodies against Tcz. On the other hand, lower serum trough Tcz concentrations (below 1 mg/L) were found in several patients(85). The influence of immunogenicity might have been underestimated in this study because the assay to detect anti-Tcz antibodies is a drug sensitive one, meaning that only ADA exceeding Tcz concentration will be detected(85). Recent publications evaluating the immunogenicity of Tcz confirm that the immunogenicity in RA patients under Tcz treatment is very rare(82,81,84) and similarly to our work, an Italian cohort showed that clinical response is better in patients with higher serum trough Tcz levels (10 μg/ml) than those with lower levels(84).

With all the above exposed data, there are no doubts about the relationship between clinical outcomes and drug immunogenicity. Nevertheless, no plausible explanation for the partial clinical response reported in some ATI+ patients is found. In fact, in our previous work in rheumatic patients under Ifx treatment, 24% of RA and 17.6% of SpA patients were responders with ATI+(72,73). This fact motivated our research group to investigate whether the timing of ATI appearance and/or drug disappearance between 2 infusions correlated to clinical outcomes. In 11 ATI+ patients

with RA, we saw that the effect of Ifx was closely correlated to the timing of the drug disappearance (related to ATI detection) between infusions(103). Another relevant finding was that higher ATI levels were found in patients with earlier drug clearance between 2 infusions. It has to be clarified that these findings were seen in a small cohort of patients but contribute to partially understand why not all ATI+ patients have poor clinical response(103).

Another important aspect is whether immunogenicity is an early event that can only be detected in the first months of treatment or whether it can occur at any time during therapy. In our patients, ATI detection occurred mainly in the first year (Mdn of 16 weeks in RA and 44 in SpA) but in some patients, ATI detection was delayed for >1 year and in SpA patients ATI was detected later than in RA patients. This may be due in part because dose of Ifx is different in both diseases and in previous studies it has been described that ATI detection is inversely correlated to the dose(72,73).

Association between immunogenicity and concomitant therapy with MTX

Several publications have demonstrated the effect of the immunosupresive drugs such as MTX or AZA on the immunogenicity in patients under biologics(5,45–48,71–73,93,105–107). The first study emerging on this topic involved RA patients under Ifx treatment, and it described that MTX has a synergistic effect(5). Similar findings have been noted in rheumatic patients treated with other TNFi such as Ada, Goli(47,106,107). In our cohort of RA patients, we saw that the drug survival was longer in patients with concomitant therapy and in the subgroup of ATI+ patients with RA, the antibody titers were lower in patients taking MTX(72).

However, the role of MTX in SpA is not clear because it is known that DMARDs have no effect on axial involvement(45,48,73,109). In our study of SpA patients treated with Ifx, concomitant treatment with MTX not only showed that frequency of ATI was lower, but also that in the subgroup of ATI+ patients, ATI titers were lower and detection occurred later than patients under monotherapy(73). The

effect of MTX in preventing the ADA development is not completely understood because it is not clear whether this effect is related to a synergistic or an anti-immunogenic effect.

Further publications have demonstrated that the effect of DMARDs such as MTX preventing ADA development is dose-dependent(46,50). Krieckaert et al. demonstrated that the probability to detect ADA is lower for patients with higher MTX dose than in patients under monotherapy or with lower MTX dose(50). Another publication including RA and PsA patients assessed the effect of MTX and other DMARDs on serum Ada trough concentrations(46). They observed that Ada trough concentrations are the highest in patients receiving MTX, (with or without other DMARDs) and patients under Ada monotherapy had the lowest serum concentrations. Although MTX seems to have the strongest influence on Ada trough concentrations, other DMARDs also seem to have a beneficial effect on drug levels(46).

Association between immunogenicity and adverse events

ADA related adverse drug reactions may appear with TNFi. The most frequent ADA-related side effects are infusion reactions, mainly with Ifx and Rtx, and injection reactions to subcutaneously administered drugs(71,93,104,110,111,113,152–155). Most mild reactions and injection side reactions are solved within 24 hours and can be treated with anti-histaminic drugs and/or corticosteroids(111). Severe reactions have been described with Ifx(111). The close correlation between infusion related reactions and ATI levels, in patients under Ifx, has been described in several studies including ours(49,71,73,96,155).

Local injection reactions are frequently seen in patients who receive a subcutaneously biological drug. The relation to antidrug antibody formation is, however, unclear. In some patients treated subcutaneously with biological drugs a systemic response is observed, but little information is available on the clinical effects of chronic immune-complex formation in these patients(111). The immunoglobulin IgG4 is an IgG isotype that has been described by some authors as potentially causing transient sensitization that leads to signs and symptoms comparable with those induced

by IgE-mediated reactions; initially termed as IgG short-term sensitizing(156). A previous study showed the presence of IgG4 anti-Etn in 13% of patients with injection-site reactions, which may predict a lower response to Etn therapy in patients(156).

Usefulness of monitoring early drug levels

A very important issue for clinicians is to find predictors of good clinical response at baseline or during the induction phase of the biopharmaceuticals. Patients with rheumatic diseases have an important disability when the disease is active and it is crucial to find the best therapeutic target for each patient as soon as possible to avoid inefficient therapies. Some authors have described that monitoring serum drug levels at early stages of the biological therapy can be related with future clinical outcomes and with ADA detection(71,94,104,119,121,148). Bendtzen et al. were the first to find that low Ifx levels at 1.5 months predicted antibody development and subsequent treatment failure in a cohort of 106 RA patients under Ifx therapy(104). A French group analysing a RA and SpA cohort observed that as early as week 2, Ifx levels were lower in patients who were later classified as ATI+(71). For other TNFi such as Ada or Etn, the correlation between the initial drug levels and the clinical response has also been demonstrated(44,119).

Other studies try to combine the monitoring of early drug levels with the initial clinical evaluation. Kobayashi et al. observed in a cohort of ulcerative colitis (UC) that early Ifx levels (at week 2), in combination with clinical evaluation are useful to predict short-long-term outcomes(120). In a small cohort of 57 RA patients treated with Ifx it was found that the combination of DAS28 at week 6 of therapy with Ifx trough levels could be predictors of clinical response at 6 months(121).

It is not clear why patients under Ifx therapy receiving the same dose at induction phase (adjusted by weight) have wide differences in serum trough drug levels. Our group demonstrated that early ATI production is detected in some patients with low levels at induction phase. Moreover, immune-complexes formed by ATI and Ifx as early as week 2 of treatment can be detected using drug tolerant assays(61,63,65). On the other hand, we defined a cut-off at early stages to predict ATI development and clinical

response during the first year of Ifx therapy using ROC analysis. Our predictive cut-off for Ifx is very similar to the one defined by Kobayasi in patients with IBD(120). These findings indicate that serum trough drug levels monitoring could be a useful tool to define which patients are more prone to have a poor clinical response.

Therapeutic strategies based on monitoring serum drug and ADA levels

In clinical practice, it is not uncommon for patients under biologic therapy like TNFi to experience a primary or secondary inefficacy and require switching to another biologic. In the guidelines, all options are allowed to switch to a second TNFi or change the mechanism of action(122,157,158). It is also crucial to consider that not all rheumatic pathologies have the same therapeutic options and before making hasty decisions, the best treatment for each patient should be considered. This fact motivated some authors to investigate if the immunogenicity against a first TNFi may affect the clinical response to a second TNFi. In a dutch RA cohort who received a first TNFi as Ifx or Ada and switched to Etn, it was observed that the clinical improvement in switchers with antibodies against the first TNFi was similar than TNF naïve patients(123). But switchers without antibodies to the first TNFi had a lower delta-DAS28 than TNFi naive and switchers with previous antibodies(123). Similar findings were confirmed by our group in SpA patients who switched to a second TNFi(118). These data suggest that for patients who fail due to inefficacy, a switch to a second TNFi seems to be a reasonable option; though patients with detectable drug levels who fail are mainly primary failures, the TNF might not be the most relevant cytokine mediating their pathology(118).

When less therapeutic options are available, the use of intensifications of biological drugs is more frequent(47,73,96,141,147). Bartelds et al. increased the Ada administration (40 mg sc weekly) in around 20% of RA patients due to inefficacy(47). Only 6 out of 20 ADA+ patients neutralized the antibodies after therapy intensifications but this strategy did not condition a relevant clinical improvement(47). In our cohort of RA and SpA patients under Ifx treatment, the need of therapy intensifications was more

frequent in ATI positive patients(73,96). In a previous study, it was seen that RA patients under Ifx frequently needed therapy intensifications but this modifications were not always enough to control the disease activity(159). When we investigated the efficacy of Ifx intensifications in a RA cohort under Ifx, stratifying by drug and/or ATI levels, similar findings to the previous study were found(141). These data suggest that therapy intensification of Ifx is limited in patients with inefficacy to the drug and the response is independent of the circulating drug levels(141).

Once the correlation between drug levels and clinical response has been demonstrated, it would be very useful to know if there is a therapeutic range associated with a good clinical response. There are two studies from a Dutch group that define a therapeutic range for Ada in rheumatic patients(70,160). These studies are carried out in RA and PsA cohort, but both find that a therapeutic range between 5-8 µg/ml was associated with a good control of the disease activity(70,160). More limited data has been published about Goli due to a smaller sample size(102,89). In our article, Goli treated patients were categorized into quartiles depending on drug levels and clinical response was investigated(102). We observed that most non responders had serum Goli levels under 0.5 mg/L(102). Other publications propose a cut off for Ada or Etn to discriminate the capacity of remission(74,161). Although the cut off is similar in these two studies, no data has been published to confirm a concentration range. For Ifx in IBD patients there is a therapeutic range defined between 3-7 µg/ml(162) but for rheumatic diseases, there is not a clear therapeutic range. Previous publications have classified Ifx levels into high, medium and low levels but there is no consensus to establish limits in each of them(108,147,163).

Some algorithms have been proposed to implement the use of drug and/or ADA levels monitoring in clinical practise(18,127,129,130,147,162,100). All these algorithms aim to improve the therapeutic decisions in clinical practice with accessory tools such as the monitoring of serum drug levels and/or ADA(18,127,129,130,147,162,100). Most of them correlate the clinical outcome (responder vs non responder) to serum drug concentrations (low, medium and high levels) and ADA status. To follow a therapeutic strategy based on each situation it is recommended to switch to another TNFi, switching to another biologic with a different mechanism of action or optimization strategies (increase or decrease the therapy regimen)(18,127,129,130,147,162,100). These

algorithms have not had much success in the field of rheumatology for several reasons: the lack of consensus to classify the levels into high, medium and low and the lack of prospective studies in rheumatic diseases designed for this purpose. In contrast, there exists a useful algorithm (TAXIT algorithm) for IBD patients treated with Ifx that has been obtained from a prospective observational study(162). In this algorithm, a therapeutic range (3-7 µg/ml) has been defined where no dose adaptation is required(162).

Utility of TDM on optimization strategies

In the last years, the use of tapering strategies is more and more frequent in rheumatic patients under biological drug treatment with a sustained control of the disease activity(131,133–135,137–140,142–145,164–171). With the accumulated experience over more than 15 years using biological drugs, our knowledge about these drugs has substantially increased in efficacy as well as in safety. Currently, it is clear when a biologic therapy should be initiated but it is unknown when it can be stopped. Several publications have emerged providing information on discontinuation or tapering strategies in RA or SpA patients(131,134,135,137–140,142–145,164–170,172–177,171). Although some articles targeted to analyze this topic are heterogeneous and use different clinical outcomes, most of them conclude that in order to perform drug tapering in established RA and SpA patients, low disease activity must be sustained. The heterogeneity of the studies is very evident in our review over discontinuation or tapering in SpA patients: after reviewing 763 studies, only 13 articles fulfilled the criteria to be included(142). In this review, we confirmed that tapering strategy is successful in most SpA patients with a sustained LDA but discontinuation strategy is not recommended because it associates with a high number of flares(142).

Even though more and more publications are emerging on this topic, rheumatologists are concerned about incidence of flares in patients under a tapering strategy and how to control them. Our group was the first one to compare long-term clinical outcomes in two cohorts of RA and SpA patients: one under a tapering strategy and other under a standard therapy regimen(143,144). In both publications, we observed

that the control of the disease activity is similar in both cohorts. In addition, the incidence of flares was comparable between cohorts and most of them were controlled with adjustments in administration interval(143,144). A progressive decrease of serum drug levels was observed during the tapering strategy, enough to maintain patients in LDA, suggesting that patients with LDA do need lower drug dose than patients with active disease. At the end of both studies, a higher number of ADA positive patients were detected in the tapering group (not significant for RA patients) but we could not demonstrate a correlation with the incidence of flares(143,144). This fact can be due to the small number of ADA positive patients in our studies(143,144).

Economic repercussion of TDM

The treatment with biopharmaceuticals represents a significant expense in our health system for several reasons, not only for their costs but also because their use is increasing and these treatments rarely discontinue. This issue involves that it is necessary to search feasible therapeutic strategies that allow our system to be sustainable. As we mentioned above, tapering strategies of biological drugs are more and more frequently used and can lead to relevant savings.

In a cohort of 51 RA patients with Ifx, Van der Maas et al. observed that dose titration was feasible in patients with inactive disease and also demonstrated important cost savings with this strategy(167). Recently, two randomized controlled trials on tapering of Ada and Etn in RA patients performed in daily clinical practice have been published(139,140). Both clinical trials conclude that tapering is feasible in RA patients without impacting structural damage progression or the appearance of flares(139,140). To our knowledge, until our publication, no study comparing the same RA and SpA cohorts before and after the tapering strategy has been published(145). Moreover, the mentioned papers comprise only disease activity guided dose reduction (including radiographic progression and flares), none of them taking drug levels into account(139,140). In our cohort, we could see that patients with good response to a TNFi treatment may successfully respond to a tapering strategy, with subsequent diminished treatment costs(145). The routine monitoring of circulating drug levels can

be a useful tool that allows the optimization of drug administration over time, avoiding erroneous tapering strategies(145). Tailored treatment options will support patient confidence and treatment compliance, as well as providing support to health care systems, allowing them to spread their budgets over many more patients because of a more rational use.

Our hospital was the first in Spain to use the serum drug and ADA levels monitoring in clinical practice. Prior to these determinations, when a therapeutic failure was detected, clinicians usually made empirical decisions, intensifying the current therapy regimen, switching to another TNFi or different class of drug. The disadvantage of choosing new biological therapies based only on clinical parameters is that it can lead to erroneous therapeutic decisions with the consequent clinical worsening of patients and the increase of financial costs. In our department, the optimization protocol for biolopharmaceuticals takes clinical (DAS28, EULAR response, BASDAI, ASDAS), serological (ESR, CRP) and structural (radiographic changes and ultrasonography) parameters into account and also includes the determination of serum drug and/or ADA levels.

In the chapter 4, we show the important cost reduction detected in our hospital after tapering strategies of biologics(145,146). Serum drug and/or ADA determinations offer the possibility to tailor therapy according to individual needs and it aims at reducing delays in effective treatment or modifying treatment strategies. In the article 13, costs per annual average-patient diagnosed with rheumatic disease show a pronounced decrease in two different periods: 2010-11 (-5%) and 2012-2013 (-13%)(146). This reduction in cost occurs despite the fact that data regarding average-dispensed-patients for the period 2010-2011 is similar, and that for the period 2012-2013 it increases from 416-452 months(146). The implementation of the Royal Decree-Law 4/2010 enforced in June 2010, resulted in a decrease of PTR of 7.5% for this type of medication. This Royal Decree-Law is the main cause for the decrease in costs in the period from 2010-2011(146). However, the decline in costs for the period 2012-2013 can be mainly explained by the optimization of treatments with dose medications by adjusting dosage and intervals of administration(146).

Important savings on biologics have been seen in other studies about optimization from our group, although this issue was not the main objective(143–146).

In our work comparing RA patients under a tapering strategy versus standard therapy regimen, we have observed a significantly lower quantity of the administered drug in the tapering group as compared to the standard therapy regimen, with an interval elongation of approximately 33% in Ifx, 53% in Ada and 53% in Etn(143). In our cohort of SpA patients under a tapering strategy comparing with a SpA cohort from the Netherlands under a standard therapy regimen, the tapering group showed an overall reduction of the administered drug at the end of the study(144). The dose reduction for Ifx was 22% and the interval was extended to 28.7%. The dose reduction was 45.2% for Ada and 51.5% for Etn(144).

Pascual-Salcedo et al. investigated the same RA cohort in low disease activity in two different periods: a period with standard therapy regimen and a period under a tapering strategy(145). The main aim of this study was to analyze whether the clinical activity remains stable after a tapering strategy of TNFi in patients with LDA and to evaluate the potential benefit of this strategy on the treatment cost(145). Finally, it was concluded that the performance of a tapering strategy in our cohort of RA patients yielded considerable savings(145). These reduction of costs were similar to what has been described by others studies of drug tapering in RA(66,139,140,167). All this evidence on this topic demonstrates that clinicians are aware of having a sustainable health system providing the best possible care and treatment to their patients. In order to make the most accurate decisions, objective tools are needed to avoid undesirable clinical outcomes that have a direct and negative impact on the patient and, secondarily, an increase in health costs.

Future

This field of research still has many open fronts and its implementation in the clinic is currently little exploited. What needs to be done to make this tool shift from research to clinic? Firstly, there should be a consensus on the assays to measure drug and ADA levels between the different researching groups. At the moment, there is a European working group (MAGE), of which we are part that has targeted this issue as one of its priorities.

Nowadays, there is abundant information on the association between drug and/or ADA levels with clinical outcomes from different unicenter studies and most of them observational and/or retrospectives. Multicentre prospective studies are necessary to address the following issues: to find an optimal therapeutic range for each drug in the different pathologies, to make a clinic algorithm based on the therapeutic range and finally to demonstrate that working based on TDM is more accurate and effective than just with only clinical tools.

Additionally, the role of monitoring serum drug and/or ADA levels in remission or low-activity patients undergoing optimization therapies is not well defined. Until the moment, all studies have included small observational studies from which it is difficult to draw robust conclusions. This type of therapeutic strategy is being increasingly used and in the coming years we will probably get more information about the utility of serum drug and/or ADA levels in this field.

The current medicine tends to be personalized in order to make a more targeted and accurate treatment for each patient. This fact motivates that there is increasing research to define more clinical tools to identify different profiles of patients. The TDM has a main objective to follow this tendency and to ensure more effective therapeutic strategies in rheumatic patients under biological therapy.

CONCLUSIONS

English

I. Lower serum Infliximab levels and the development of antibodies to Infliximab are associated with a poor clinical response, an earlier therapy discontinuation and an increased incidence of adverse effects in Rheumatoid Arthritis and Spondyloarthritis patients treated with Infliximab.

II. The concomitant use of Methotrexate along with tumor necrosis factor inhibitors is useful to prevent anti-drug antibody development in Rheumatoid Arthritis and Spondyloarthritis patients.

III. The effect of Infliximab treatment in patients with anti-drug antibodies has a relationship with the timing of the drug disappearance and anti-drug antibodies appearance between infusions, each resulting in different clinical consequences.

IV. Serum Infliximab levels at the induction phase of the treatment might predict the group of patients that are more prone to develop antibodies to Infliximab and therefore, have a worse clinical course during the first year of therapy.

V. The Tocilizumab standard regimen results in a wide variety of serum Tocilizumab trough concentrations between patients, and target binding seems to provide a better explanation for these variations than immunogenicity. Serum Tocilizumab levels are associated with C-reactive protein levels and clinical response.

VI. Serum Golimumab levels are associated with the clinical activity and acute phase reactants in Rheumatoid Arthritis patients during the first year of therapy.

VII. The presence of antidrug-antibodies against the first tumor necrosis factor inhibitor is an influencing factor for the response to a second tumor necrosis factor inhibitor. The study of the immunogenicity in the biological treatment failure may help to predict the response to a second biological drug in Spondyloarthritis.

VIII. Increasing the Infliximab dose in non-responding patients is expensive and was unsuccessful in our cohort of Rheumatoid Arthritis patients

IX. Performing a tapering strategy in Rheumatoid Arthritis and Spondyloarthritis patients with low disease activity, along with a tight clinical control of the drug/anti-drug antibody levels, seems to be feasible and a cost-effective strategy without causing relevant clinical impact.

Spanish

I. La detección de bajos niveles de Infliximab y el desarrollo de anticuerpos anti-fármaco se asocia con una peor respuesta clínica, aumento de frecuencia de abandono del tratamiento y una mayor incidencia de efectos adversos en pacientes con Artritis Reumatoide y Espondiloartritis tratados con Infliximab.

II. El uso concomitante de Metotrexato con inhibidores del factor de necrosis tumoral es útil para prevenir el desarrollo de anticuerpos anti-fármacos en pacientes con Artritis Reumatoide y Espondiloartritis.

III. El efecto de Infliximab en pacientes que ya han desarrollado anticuerpos anti-fármaco se correlaciona con el momento de la desaparición del fármaco y la

aparición de anticuerpos anti-fármacos entre las infusiones, condicionando diferencias en la respuesta clínica.

IV. La monitorización de los niveles séricos de Infliximab en etapas tempranas puede predecir el grupo de pacientes más propensos a desarrollar anticuerpos contra Infliximab y tener un peor curso clínico durante el primer año de tratamiento.

V. La administración de Tocilizumab a dosis estándar da lugar a una amplia variedad de concentraciones séricas del fármaco entre los pacientes, y la unión al objetivo parece proporcionar una mejor explicación de estas variaciones que la inmunogenicidad. Los niveles séricos de Tocilizumab se asocian con niveles de proteína C reactiva y respuesta clínica.

VI. Los niveles séricos de Golimumab se asocian con la actividad clínica y los reactantes de fase aguda en pacientes con Artritis Reumatoide durante el primer año de tratamiento.

VII. La presencia de anticuerpos anti-fármaco contra el primer inhibidor del factor de necrosis tumoral influye en la respuesta clínica a un segundo inhibidor del factor de necrosis tumoral. El estudio de la inmunogenicidad en el fracaso del tratamiento biológico puede ayudar a predecir la respuesta a un segundo tratamiento biológico en la Espondiloartritis.

VIII. La estrategia de intensificación de dosis con Infliximab es costosa y no tuvo éxito en nuestra cohorte de pacientes con Artritis Reumatoide, independientemente de los niveles de fármaco en circulación antes de la escalada de la dosis.

IX. La estrategia de optimización de biológicos en pacientes con Artritis Reumatoide y Espondiloartritis en baja actividad mantenida, realizando un control clínico estrecho junto con la monitorización de niveles séricos de fármaco/anticuerpos anti-fármaco, parece una estrategia factible y rentable sin ocasionar un impacto clínico relevante.

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